

Who will make the trip to Mars?

President George Bush's plans for Mars will disappoint the enthusiasts, but they will not send the value of the US dollar into decline, for which everybody should be thankful.

LAST Thursday's celebration, at the Air and Space Museum in Washington, of the twentieth anniversary of Apollo 11's landing on the Moon found President George Bush in typical laconic and noncommittal speech. "This vision thing" — Bush's phrase in another context for uplifting views of what the future holds — was more evident in what three lunar astronauts had to say than in his own address. True, there were some positive declarations — "We must commit ourselves anew to a sustained program of manned exploration of the Solar System and, yes, the permanent settlement of space", for example — but their delivery was flat. Worse, there was no timetable. Bush properly made it plain that that decision rests with US Congress and its capacity to appropriate the funds. Both Bush and the Congress now know that getting to Mars is not a technical but a financial problem.

Comparisons with President John F. Kennedy's well-sounding launch of the Apollo programme are inevitable, inevitably invidious, but illuminating as well. Kennedy's goal was an adventure that would demonstrate the quality of US technology and also set a milestone in the track of human history. Apollo was a huge risk. When the United States was still smarting from the unexpected success of the Soviet Sputniks, it was a firm commitment to reach the Moon within a decade. What would have happened if the technology had failed? Or if the cost of making it work had exceeded the estimated cost of \$20,000 million? Kennedy implied that the United States was strong enough to take the risk and to pay whatever bills accrued. Early in the 1960s, that was true. Even the commitment to Apollo made the people of the United States feel good. The success of the project less than a decade later would have been permanently uplifting if, by then, the US commitment in South Vietnam had not proved socially divisive and financially debilitating.

Sadly, as Bush knows, things are different now. Japanese technology now has the edge, although not decisively, while the US federal budget deficit means that the Congress has hardly any room in which to find the cost of the few hundred thousand million dollars that an expedition to Mars will cost. If Bush last Thursday had said that an "American will walk on Mars by 20XX", he would have instantly have undermined the patient efforts of Mr Alan Greenspan and the Federal Reserve to walk the tightrope between inflation and recession and would have sent the value of the US dollar tumbling. Bush, in

the circumstances, did the most he could: there will be US astronauts on Mars when the cost has been calculated accurately and when the United States can afford it.

The disappointment in last week's declaration is, or should be, more subtle. By citing only historical inevitability as the reason why US citizens should go to Mars, Bush has begged a long list of questions. What part will be played in this new adventure by the Soviet Union and, as the decades roll by, by Japan? Will the technical demands of the enterprise so stiffen and stimulate now-bankrupt public education in the United States as to meet the needs of civilian industry for skill, or alternatively further divert what skill there is from useful and profitable enterprises? And what is it for?

The plain truth is that there are less risky, cheaper and more productive alternatives to what is now proposed. First, just within grasp, is the Hubble Space Telescope, still on the ground at Los Angeles, which will certainly clarify and may well transform our picture of how the Universe is constructed. Does it make sense that such a potential transformant of the human imagination should continue to wait on the vagaries of the shuttle's schedule? And what of the imaginative proposal for an international centre in astrophysics (see *Nature* 339, 574; 22 June 1989) being canvassed by astronomers and space scientists from the United States, the Soviet Union and elsewhere? Instruments will tell us what it is like to walk on Mars long before people can get there: would it not be more valuable to know what the whole Universe is like? Sober enterprises like these would help enormously to restore the world's faith in science and technology as a means to understanding. □

Europe and Japan

The British House of Lords has made a perceptive comment on relations between Europe and Japan.

EUROPE, hugging itself with pleasure that it will be a true common market by the end of 1992, is nevertheless scared stiff of the prospect of ruinous technological competition with Japan. So much is clear from the ways in which the European Communities (EC) and individual member states have sought over the years to protect themselves. Many European states have enacted compulsory quotas



on imports from Japan while others have persuaded Japanese motor manufacturers to accept 'voluntary' quotas on their exports. From time to time, European governments use more devious means to prevent their people from buying Japanese goods: the French government's diversion for 'inspection' of imported video-recorders to warehouses in provincial Poitiers remains a triumph of ingenuity among non-tariff restraints on trade — but, as events have shown, an ineffectual one. EC's more formal restriction of the import of Japanese goods by anti-dumping measures are even less easily justifiable and should be, as Japan asks, adjudicated by the General Agreement on Tariffs and Trade (GATT). This is the small change of bad international relations, but worrying at the outset of the European drive for self-cohesion.

That is the incentive for a study (now published) by the Select Committee on the European Communities of the British House of Lords. When, in the early 1970s, soon after British membership of EC, it became plain that the spate of European regulation and legislation would overwhelm the House of Commons, Lord Ashby (previously Eric Ashby, researcher and academic) persuaded his fellow-peers that the House of Lords should scrutinize European legislation instead. If the British government had taken the committee's advice on pollution standards, it might not now find itself compelled to meet European standards for the purity of drinking water while trying to sell the British water-supply industry to private shareholders. Now the committee has taken up the question of Europe's relations with Japan, using a somewhat narrow-minded document produced by the European Commission last year as its starting-point.

What the committee says is familiar, but none the less sobering. First, it offers an accurate diagnosis of why Europe is at a competitive disadvantage with respect to Japan in the trade for high-technology consumer goods: the Japanese manufacture goods of better quality and reliability which are innovative enough to command the pocket-books of customers. On the management of the awkward trading relationship that results, the committee argues forcefully that the European Commission should take the issue firmly in hand, abolishing the present patchwork of national and Community-wide restriction and, if necessary, applying (as is allowed) to GATT for temporary protection for vulnerable industries. The present mixture of arrangements is a recipe for contention.

For Europeans, the chilling part is the explanation of Japan's success. The quality of Japan's public education persuades one British industrialist that many of Japan's production-line workers would be welcome in his development laboratories. The information that large Japanese manufacturers regularly spend 15 per cent of their turnover on research and development is not new, but is probably the most direct demonstration of how science and technology can create prosperity: *per capita* Gross Domestic Product in Japan, which now exceeds that in the United States by 16 per cent, is two-thirds greater than in EC as a whole. In passing, the committee

notes that the Commission's applied research programmes, devised in part to simulate the pre-competitive research collaboration in which Japanese companies engage, are nowhere near as effective. The committee also flirts with the superficial notion of 'catching up', but understandably without conviction. The challenge for Europe is how to strike a better balance. □

Games British play

The re-reorganization of British civil science seems destined to come about, but only with difficulty.

QUITE why a radical proposal that five British research councils should be rolled together into one have, just now, been taken up with gusto is a matter for conjecture (see *Nature* **339**, 645; 29 June 1989). Is it that the ticking of some biological clock has reminded the British that it is a long time now since the reorganizations of 1962 (Trend), 1971 (Rothschild) and the onset of the past decade's slow attrition of 1981? But nobody in Britain expects Rome to be built or even dismantled in a day. Last week's meeting of the Advisory Board for the Research Councils (ABRC) leaves the most important details to be settled. Meanwhile, the chairman of the Science and Engineering Research Council (SERC), Dr E.W.J. (Bill) Mitchell, has circulated to his staff a document which is at once a defence against some of the criticism in the Morris report that triggered the present interest in reorganization and a cogent argument for reorganization.

All public grant-making organizations are necessarily anomalous. Their function is to disburse public money for research, but they depend primarily for expertise on the researchers who depend on them — the members of the committees who sift through research-grant applications. Mitchell is right to argue in his letter that SERC has been unfairly blamed for the turf-battle with the Medical Research Council (MRC) over the no-man's-land of biotechnology. He might have added that SERC has also won an enviable reputation for fairness among its dependants. This journal's complaint is that it should be trying harder to spend more of its income on untied research in universities. That could be easier if the five research councils were united, but he is right that such an arrangement would be nonsense if one council (MRC is the reluctant partner) is allowed to stay outside.

The solution, for ABRC and the British government, is to finesse these difficulties by experiment. Hitherto, reorganization has been taken to imply the abolition of the present structure and its replacement by another. Why not instead make the chairman of ABRC a full-time executive for the management of civil science, make the council into a source of strategic advice and let the research councils work out the best way of living with each other productively? That way, the utility of reorganization would be tested against need. Too many of the reorganizations of the past have been nullified by their abstractness. □

Bush calls for missions to the Moon and Mars

■ Congress says budget not available

■ Space station a stepping stone to planets

Washington

COMMEMORATING the twentieth anniversary of the day when the Apollo 11 astronauts made the first ever landing on the Moon, US President George Bush proposed in Washington last week to "commit ourselves anew to a sustained programme of manned exploration of the Solar System and ... the permanent settlement of space". He instructed the National Space Council, headed by Vice-President Dan Quayle, to determine "money, manpower and materials, the feasibility of international cooperation" and "report back to me as soon as possible with concrete recommendations to chart a new and continuing course to the Moon and Mars and beyond".

But these words, delivered on the steps of the Smithsonian Institution's National Air and Space Museum to a large gathering of astronauts, were no sooner out of the president's mouth than he was attacked by congressmen for failing to explain where the money for a massive manned space programme could come from. "There is no such thing as a free launch", said House of Representatives majority leader Richard A. Gephardt (Democrat, Missouri), "We got more words, I don't hear any 'how to do it', that's what we really need". Even as the president spoke, the National Aeronautics and Space Administration (NASA) was fighting to keep Congress from cutting \$700 million from the 1990 budget for the manned space station Freedom, in a House of Representatives debate that eventually appropriated \$12,300 million for NASA, \$1,000 million less than the White House had asked for.

A major announcement from the president was widely expected, given the symbolic significance of the twentieth anniversary of the lunar landing and the weeks of rumour that the vice-president had developed plans for a major commitment to a mission to the Moon without consulting senior administration budget officials.

At the anniversary celebration, the astronauts who spoke before the president clearly hoped that he was about to echo President John F. Kennedy's 1961 speech that began the race to the Moon.

"Perhaps we are at the starting line [for a planetary initiative]", said astronaut Edwin "Buzz" Aldrin, one of the three Apollo 11 crew. But the president avoided going as far as Kennedy had, while giving manned space exploration the strongest

support that NASA had heard in years.

NASA's Office of Exploration is already charged with assessing the technology needs for Moon and Mars missions. The president's announcement is likely to hasten its requests for funds and to direct it to a three-stage approach; with, as the president said, the space station Freedom for the coming decade, permanent occupation of the Moon in the next century, followed by a manned mission to Mars. Other plans under consideration, including a direct flight to Mars from a more distant space station, appear to have been rejected in favour of gaining experience at a lunar base before an attempt at Mars.

To fulfil these goals stretching into the next century, NASA would have to begin by developing a new class of heavy-lift boosters capable of delivering 300,000-lb payloads to the space station. These boosters would carry the materials necessary for in-orbit assembly of the spacecraft that would shuttle back and forth between the Moon and the space station. A Moon base might consist of no more than a small science laboratory; or it might be developed into a full-scale industrial base if lunar soil can be processed to extract oxygen to power spacecraft. If that proves possible, then a lunar base makes sense as a stepping-stone to the planets. Otherwise, it would almost certainly be cheaper to go direct to Mars from a space station in high Earth orbit.

No-one has a definite price for manned exploration of the planets, but a figure of \$100 thousand million to reach the Moon and \$400 thousand million for the Moon and Mars have been given by NASA

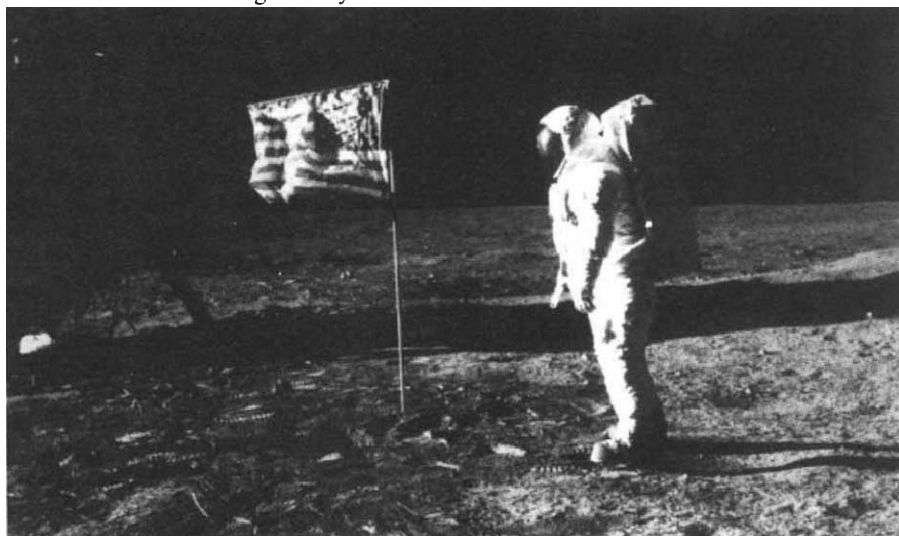
researchers. Over a 30-year period, accommodating these extra programmes would require NASA's present \$11 thousand million a year budget to be increased two- to threefold. But even at \$30 thousand million a year, NASA's budget would be a little less, as a percentage of the total federal budget, than at the height of the Apollo project.

But as Gephardt pointed out, times have changed since the days of the Apollo programme; the rapid growth of the 1960s has gone and the problems of the trade deficit and foreign competition mean that the "\$25-\$50 thousand million a year" needed for a full manned programme could not be found in a constrained budget.

Despite the president's rhetoric — "the only footprints on the Moon are American footprints" and "it is America's destiny to lead" — Congress has worldly matters to consider. At a hearing of the Senate subcommittee on science, technology and space earlier the same week, Senator Al Gore (Democrat, Tennessee), the chairman, pointed out that in the race for space of the 1990s the goal is no longer military superiority or technological prowess but "market share, new products, new industries" in an increasingly crowded market place for space services. Gore asked for a "viable budget" before "another spectacular".

Even the role of the space station, the first step in any manned return to space, seems still unclear. Bush said the "space station will also serve as a stepping-stone to the most important planet in the Solar System, planet Earth..." and went on to back the use of space to monitor the Earth's environment. But the \$30 thousand million space station, already delayed by budget uncertainties, is designed primarily as a laboratory for microgravity research. Its low, equatorial orbit makes it unsuitable for most Earth observation and its initial configuration is not designed for the factory assembly of Moon-bound rockets.

Alun Anderson



Edwin "Buzz" Aldrin on the Moon, 20 July 1969.

Protest movement in Japan

Tokyo

CHINESE students in Japan have formed an underground movement to keep alive the spirit of the pro-democracy demonstrations crushed in Beijing's Tiananmen Square massacre on 4 June. The Tokyo-based movement represents the first concerted effort by Chinese students in Japan to form an active organized resistance to Beijing's crackdown against anti-government activists.

Japan, with nearly 30,000 students from the People's Republic of China, is the second largest base for Chinese foreign students after the United States. Chinese students in Japan have in the past played an influential role in shaping Chinese politics. A classic example is Lu Xun, who as a medical student in Japan before the Second World War wrote several books on the shortcomings of China, the most famous of which is *The True Story of Ah Q*. His writings had a great influence on reformists before the communist revolution of 1949 and were adopted as communist teachings by Mao Tse Tung in the 1950s.

The identities of participants in the new underground movement have been kept largely secret, but this month the first signs of it emerged in Tokyo. A group calling itself "Chinese students' association for furthering democracy" published the first edition of *Democratic China*, a magazine calling for more democracy, freedom and the overthrow of China's current leadership.

The 72-page magazine carries gruesome black-and-white pictures of the carnage on the streets of Beijing in early June, editorials lambasting Beijing's leaders as "butchers" and articles detailing resistance movements of other overseas Chinese students, mainly in the United States. It is not clear how many Chinese students are involved in writing and publishing the magazine but Japanese sources close to the underground movement said it had at least 20 active members. About 3,000 copies of the magazine have been sold at bookstores in Japan that deal with Chinese literature. A Japanese source said one of the current leaders of the movement is Yang Zhongmei, a student whose name appears alone on the front pages of *Democratic China*.

Beijing has branded all pro-democracy student activists "counter-revolutionaries" and has mounted nationwide manhunts within China to arrest them. Chinese students in Japan say that speaking out against the government could limit their chances of being allowed to stay abroad, cause persecution of their families and bring them punishment on their return.

"China is far too unstable at the moment. It is impossible to tell what the government will do next", said one self-

paying Chinese student from Shanghai who had nothing to do with the underground movement. "Chinese secret police are very active in Japan. There have been some new, suspicious students at our school. The best thing to do is not talk to Chinese here about politics." China's embassy in Tokyo maintains that conditions for Chinese students in Japan have not changed. An official in the overseas study department of the embassy says there have been no cases so far of "counter-revolutionary" students being sent back to

China. But the official said the number of self-paying students arriving from China has drastically decreased since 20 June when Beijing imposed stringent new restrictions on overseas travel.

The new regulations in Beijing require people going abroad to obtain "positive vetting" from their supervisor, university, school or work unit certifying that they did not take part in "counter-revolutionary" activities. The restriction has made many people wary of applying to leave the country, "but it is mainly a bureaucratic problem which should ease over the next few months", the Chinese official said.

David Swinbanks

... and in the United States

Washington

RUMOURS of spying and surveillance by agents of the Chinese government are rife among Chinese students and academics in the United States. At a rally on Capitol Hill last week organized by a newly formed pro-democracy group, the National Coordination Committee on Chinese Student Affairs, students said that many had received warning telephone calls and even death threats from unidentified callers who they suspect to be Chinese embassy staff or government agents. Gong Xiaoxia, a graduate student at Harvard University and one of the organizers of a pro-democracy group, the China Information Center, based near Boston, says the centre is being watched. The office has been broken into and students have received telephone calls warning them that they are doing "very dangerous things".

The Chinese Embassy in Washington denies any involvement, saying in an official memorandum that "it is our consistent position to oppose any kind of monitoring, harassing or intimidating of overseas Chinese students, directly or indirectly". But neither US congressmen nor students give much credence to the words of embassy staff.

The Chinese government has stopped making daily announcements of arrests and sentencings but has not stopped rounding up "counter-revolutionaries". Last week it issued a new list of 48 most wanted people. Among those arrested last week was Yang Wei, a former graduate student in biochemistry at the University of Arizona, who had been released from prison in January after serving a two-year sentence for pro-democracy activities. Congress passed a resolution calling for his release in 1987 when he became the first Chinese studying in the United States to be arrested for political activities. The human rights group Asia Watch also reported this week the arrests of Liu Fang, a student at Beijing Medical University,

and Zhou Duo, an economist with Stone Corporation, China's largest computer company.

Various bills introduced to allow Chinese students and academics to extend their stays in the United States were examined last week by the House of Representatives subcommittee on immigration, refugees and international law. The most strongly supported bill, with more than 240 sponsors, is similar to that passed unanimously by the Senate last week (see *Nature* 340, 174; 20 July 1989) and would waive the J-1 visa requirement that students return to China for at least two years when their studies are completed. A bill sponsored by the committee's chairman Bruce Morrison (Democrat, Connecticut) would create a new legal status called "temporary protected status" which could apply to threatened people of any nationality. Both bills were approved by the subcommittee but are unlikely to be voted on before the summer recess in two weeks time.

The committee did not approve a bill which would make it easier for Chinese to get political asylum and another which would allow an increase in the number of immigrants from Hong Kong.

Congressmen also attacked the one-year visa extension granted to Chinese students by President George Bush immediately after the Tiananmen Square killings. Only four students have taken up the president's offer because it requires a formal application that may mark people for punishment when they eventually go home. But Paul Virtue, acting general counsel of the Immigration and Naturalisation Service, said the administration opposed further measures and that the current one-year visa extension was a success. He argued that a waiver of the two-year residency requirement would benefit those who opposed democratic reforms as well as those who supported them, and it could jeopardize future exchange programmes.

Christine McGourty

HEALTH

Transfer of responsibility

Washington

OUT of concern that the US Department of Energy (DoE)'s mandate to construct nuclear weapons conflicts with its responsibility to track the health effects of building bombs, Congress is poised to give the latter task to health agencies instead. This week, amendments transferring DoE's epidemiological functions to the Department of Health and Human Services (HHS) are expected to be tacked onto next year's Defense Department spending bill.

The move is prompted by recent disclosures of safety breaches and environmental contamination at several DoE facilities. Despite assurances by the new Secretary of Energy, James D. Watkins, that his recently unveiled ten-point plan to revamp DoE will bolster the agency's "accountability", Representative Lane Evans (Democrat, Illinois) says it still leaves the "fox guarding the chicken coop". Evans contends that DoE has suppressed studies linking exposure to low levels of radiation to cancer and other health problems, so DoE officials could keep the nuclear-weapons production machine up and running. The agency withheld epidemiological data from outside examiners, says Evans, not to protect national security, but to drop a "veil of secrecy" over the effects of its activities.

Evans's amendments would take away DoE's authority and budget to conduct epidemiological research on nuclear-

weapons plant workers and local residents, and give it to HHS, the agency containing the National Institutes of Health and the Centers for Disease Control. They would also set up a nine-member presidential advisory panel on radiation research that would include representatives from public interest groups, trades unions, and members from the public health departments of states with a defence nuclear facility. The results of any studies would be opened up to public scrutiny. Congressman Ron Wyden (Democrat, Oregon) plans to introduce a similar bill next month.

But Under-Secretary of Energy John C. Tuck says the health agencies already have "an extremely full plate" and that Assistant Secretary for Health James O. Mason is not keen to take over DoE's role. The National Institute for Occupational Safety and Health already sets radiation exposure standards for workers, and Congress has asked the Centers for Disease Control to evaluate whether further epidemiological studies need to be done near two of DoE's plants.

If Congress will let DoE keep its epidemiological research functions, Tuck says DoE will set up its own panel of experts from outside the agency to advise it on how to restructure its programme. DoE will also complete a \$36-million epidemiological data repository and open it up to "any qualified researcher".

Carol Ezzell

Campaign in France for democracy in China

Paris

THE presence of 6,000 journalists in Paris for the bicentenary celebrations on 14 July gave a media boost to the efforts of Chinese students campaigning for democracy in their country.

Wuer Kaixi and Li Lu, refugee leaders of the student movement, inaugurated a replica of the statue of the Goddess of Democracy which was demolished by tanks

in Tiananmen Square on 4 June.

A solemn procession of Chinese students in mourning, which led the spectacular bicentennial parade down the Champs Elysées before 34 heads of state, was greeted by a standing ovation.

An association for the Coordination of Chinese for Democracy (CCD) was set up soon after the events in Tiananmen Square to advise the 3,000 Chinese studying in

France and to maintain pressure on the Chinese government for a return to democracy. So far, says CCD president Jing Yizhong, the French government has given constructive support. Expired visas will automatically be renewed and it has been made easier for Chinese to obtain entry to France. Access to jobs has been facilitated for students unwilling to return to China, and both CCD and the French

TECHNOLOGY TRANSFER

Arianespace accused

Paris

ARIANESPACE, the consortium responsible for European commercial space launchers, has been accused of infringing international technology-transfer regulations over negotiations to sell its Viking rocket motors to Brazil. The accusation comes from US Democrat Dante Fascell, chairman of the Senate Committee on Foreign Affairs. He claims that the sale would contravene a treaty signed by the seven most industrialized nations in 1987 limiting the export of technologies which could be used to manufacture ballistic missiles (see *Nature* 339, 329; 1989).

The Brazilian telecommunications organization, Imbratel, has sent out a call for tenders to launch two Brasilsat satellites. As part of the deal, Arianespace has been asked to contribute technology to help Brazil develop its own small satellite launch vehicle (VLS).

In Paris, Arianespace says that the US reaction is both premature and inappropriate. No agreement can be signed without prior approval from the French government and, in any case, the technology transfer would take place in stages over a period of 15 years.

The Viking rocket, said a spokeswoman, should not come under the 1987 treaty. The rocket is destined for small launchers and uses liquid propellant, whereas ballistic missile rockets usually use solid fuel. The Viking technology has already been sold to India, said the spokeswoman.

A US Delta-2 rocket, made by McDonnell-Douglas, is the main competitor with Arianespace's Ariane-4 for the Brasilsat contract. At Arianespace there is a feeling that the United States is simply making trouble to help its long-abandoned commercial launch programme get back on its feet.

Peter Coles

government are discouraging students who want to return.

With a donation from couturier Yves Saint-Laurent and the Paris-Peking association, CCD has acquired an empty boutique in the centre of Paris to serve as meeting-room and headquarters. Under the slogan "We will continue", CCD says it is essential to continue to put pressure on the Chinese government, not just for Chinese students abroad but "for the whole of humanity". "When the Chinese government says it does not care about protests in the West", says Jing Yizhong, "it is lying. In fact it is very concerned about these reactions." Chinese student leaders in Paris, Cai Lin, Wuer Kaixi and Wan Dan are expected to seek asylum in the United States shortly.

Meanwhile, CCD is supporting their nomination for the Nobel Peace prize.

Peter Coles

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Chinese student leader Wuer Kaixi (left) and top intellectual dissident Yan Jiagi announced recently, in a French-television interview, their intention to set up an organization to carry on the fight for freedom in China.

Problems of security

Washington

WHEN a computer 'virus', written by Cornell University graduate student Robert Morris, traversed the Internet electronic-mail network to infect thousands of host computers (see *Nature* 336, 97; 1988), the immediate flaws in the system which permitted the tainted program's easy transmission were quickly remedied, and no permanent damage was caused. But the incident exposed general weaknesses in the security of computer networks, which the General Accounting Office (GAO), an investigative arm of the US Congress, sets out in a report issued last week. And at a hearing in Congress last week, some remedies were proposed which may alarm computer users more than the virus threat itself.

The GAO report highlights two problems: lax security at academic computer installations and a lack of established legal principles by which network intruders can be prosecuted. In the aftermath of last year's virus infection, computer managers and users have become more safety-minded (see *Nature* 336, 301; 1988), and a number of bills now being considered in Congress have provisions to extend definitions of fraud and property so as to encompass computer-security violations. But GAO goes further and recommends the establishment of a central body, under the umbrella of the White House Office of Science and Technology Policy, which would oversee security policy and take a hand in network management.

No detailed proposal is made, but the idea that changes in a local operating system, for example, would have to be submitted to a central body for scrutiny

SATELLITES

Hipparcos launch delay

London

AN electrical storm has delayed the launch of the Hipparcos, the European Space Agency (ESA)'s astrometry satellite, originally scheduled for 27 July (see *Nature* 340; 111-116, 1989). According to project director Michael Perryman, the problem stemmed not from Hipparcos but from the television satellite TVSAT-2, which shares an Ariane-4 rocket with Hipparcos. The storm broke when TVSAT-2 was powered up for electrical tests, and extra time was needed for additional tests to check for storm damage. All systems now appear to be in working order, and the mission will now blast off from the ESA Space Centre in Kourou, French Guiana, on 8 August. The TVSAT series has been dogged by problems: TVSAT-1 was launched successfully, but was effectively rendered useless because its solar panels failed to deploy. Henry Gee

and approval would not sit well with the free-thinking ethos of university computer departments.

Jim Conklin of EDUCOM, an organization that studies information and communications issues for the academic community, agrees that a combination of technical and legal action is needed to guard against future virus attacks, but warns against measures that would "kill the network" in fighting the "occasional glitch".

Although the GAO report makes the criticism that many individuals who discovered the virus had no idea to whom they should report the problem, the quick preventive action against the intrusion was in part due to network users who wrote and distributed anti-viral program patches without bothering to consult any higher authority. Over-elaborate security policies and reporting procedures, says Conklin, may stifle this kind of rapid but improvised response.

Many computer users who had cheered security violations, such as the break-in by a West German amateur into a system at Lawrence Berkeley Laboratory (see *Nature* 333, 105; 1988), as praiseworthy examples of the 'hacker' mentality changed their minds when Morris's virus threatened their own filespace. Conklin sees this change as part of a needed institutional response. The other ingredient is the creation of new legal principles, but this may not be easy. The Federal Bureau of Investigation (FBI) made little attempt to investigate the Berkeley breach: no data were stolen and no programs were destroyed, and the FBI could find no firm grounds for prosecution.

The kind of legal measures suggested by GAO and, at a House Energy and Commerce subcommittee hearing last Thursday, by John Landry of ADAPSO, a computer software and services industry association, would rely on new definitions of unauthorized access and damage to computers, so that the perpetrator of a virus which merely slowed down operations without erasing data or programs would be prosecutable. But does the spread of a virus on an open electronic mail network constitute 'unauthorized access'? And is a program that browses through a database, perhaps copying parts of it, engaged in an act of theft? The technicality of the concepts involved has discouraged tests of existing laws which might address these questions, and makes it hard to draw up sound new laws. The best that can be hoped for, according to Conklin, is that network security and legal safeguards will at least keep pace with the threats; those who operate and use the networks are as ingenious as those who would infiltrate them.

David Lindley

Youth plan takes shape

Munich

THERE can never be enough money for university research; this maxim was demonstrated once again last week by the influential West German science council Wissenschaftsrat, which greeted enthusiastically a plan to invest DM6,000 million (about \$3,150 million) over ten years to keep young researchers at universities. Mouths watered in universities all over West Germany in May when Education Minister Jürgen Möllemann (Free Democrat) announced the plan, the purpose of which is to prepare for an expected wave of retirements of university professors in the years 1995 to 2005.

The council made clear, however, that the best students can be persuaded to stay at university only if there is sufficient support for their research. So the granting agency DFG (Deutsche Forschungsgemeinschaft) should receive the second-largest share of the money after the universities themselves. DFG spokeswoman Eva-Maria Streier said that DFG would plough virtually all the money into its normal granting programmes.

Streier also said that DFG supports the council's plan to encourage more women to enter science by providing special scholarships and encouraging flexibility in career planning. Figures distributed in early July by the Federal Statistics Office in Wiesbaden underscore the need for more women in academic pursuits. Although about 40 per cent of the students at West German universities are women, only 5.1 per cent of professors are women.

The council also called for extending the so-called Fiebigger programme until about 1995. This programme gives short-term positions to young researchers until professorships become available.

An official at the Research Ministry (BMFT) sees similar problems in government research establishments such as the Max Planck Institutes and the so-called Large Research Establishments. He welcomed the programme and suggested taking advantage of the capacity of such research institutions to employ graduate students and other young researchers until there are jobs available for them in universities.

At present, many of the best students do not even apply for stipends for postgraduate research because industry can offer them more money, said Wilhelm Krull of Wissenschaftsrat.

The heads of science organizations in West Germany eagerly await a meeting on 21 September with Möllemann, Research Minister Heinz Riesenhuber, Chancellor Helmut Kohl and the heads of the *Länder* (states), when a decision is expected about the size of the programme. Until then, researchers remain sceptical about whether there will be as much money as Möllemann has promised. Steven Dickman

AIDS

US patent for Montagnier

Washington

A US PATENT for HIV-2, the second virus found to cause AIDS, has been granted to Luc Montagnier of the Institut Pasteur in Paris. The all-encompassing patent — it has a total of 48 claims — was announced last week by the US drugs company Bristol-Myers, whose subsidiary Genetics Systems Corporation holds the exclusive rights for diagnostic applications.

The granting of the patent may reopen discord between US and French AIDS researchers. Priority for the discovery of HIV-1, the virus causing the majority of AIDS cases outside Africa, was hotly contested for several years in US patent battles involving Montagnier and Robert Gallo of the US National Cancer Institute. Although Montagnier and Gallo have said the dispute did not affect the "spirit of scientific cooperation" between their two laboratories, it eventually escalated to include both the French and US governments.

In early 1987, the Institut Pasteur and the US Department of Health and Human Services signed a settlement agreeing that the antibody test kit for HIV-1 was a "joint invention", based on a chronology of AIDS discoveries (see *Nature* 326, 435; 1987). The terms of the settlement also dictated that 80 per cent of the royalties resulting from the sales of HIV (human immunodeficiency virus) antibody screening kits would be donated to the French and American AIDS Foundation, with a portion going to the World AIDS Foundation.

Montagnier's HIV-2 patent's first and broadest claim reads "A human retrovirus, wherein the retrovirus is HIV-2 in a biologically pure form". But the subsequent 47 claims cover every possible facet

of HIV-2: *in vitro* cultures, supernatants, lysates and extracts; various combinations of labelled and unlabelled viral proteins that could be used in blood tests; and specific dosages of various protein "compositions" that could be used in a potential vaccine.

The patent was filed in March 1986, and rests on work done in late 1985 and early 1986 on cultured lymphocytes from a person with AIDS from Guinea-Bissau who did not test positive on an antibody test for HIV-1. But Myron Essex from the Harvard School of Public Health was also involved in research at about the same time on a virus that was also later designated HIV-2.

Harvard University has filed patents based on work done in his laboratory which could turn out to coincide with the discoveries underlying Montagnier's patent, throwing it open to an 'interference' investigation by the US Patent Office. Essex is a consultant for Cambridge BioScience, a Massachusetts bio-technology company that has been developing screening tests for HIV-2.

The potential market for a separate diagnostic test for HIV-2 is a fraction of the \$100 million market for HIV-1. The utility of such tests is debatable: because current tests for HIV-1 antibodies identify between 50 and 90 per cent of those infected with HIV-2, a test for HIV-2 would detect only a few additional cases. Most of those infected with HIV-2 are in Africa.

Montagnier's patent issued quietly last month, with many of the central players in AIDS research and policy getting wind of it only late last week. Gallo, who along with Montagnier is also one of the directors of the World AIDS Foundation, says he is "surprised and concerned" that Mon-

tagnier's proprietary interests in HIV-2 were not disclosed when the HIV-1 settlement was reached in 1987. He says the new patent is "worth a significant discussion, if not more". Montagnier declined to comment without first reviewing his background materials.

Carol Ezzell

ROCKET LAUNCHERS

Engine problems shake Japan's space plans

Tokyo

JAPAN'S plan to build a domestically developed rocket for the launch of large satellites has suffered a serious setback. The National Space Development Agency (NASDA) says that the first launch of the H-II rocket will have to be delayed a year because of problems in the development of the cryogenic oxygen-hydrogen engine in the first stage of the rocket.

The H-II will be Japan's first domestically developed liquid-fuel rocket and will be capable of putting a 2,000-kg satellite into geostationary orbit — almost four times the payload capacity of the H-I rocket now in use. The rocket is expected to compete with those of other nations for the commercial launch of satellites.

But NASDA's contractors are having serious problems with the first-stage engine. In test runs the nickel-alloy turbine blades of the hydrogen turbopump, used for pumping the liquid hydrogen fuel, resonate and crack. This problem was not encountered with the second-stage cryogenic engine of the H-I, which also forms the second stage of the H-II, because it operates at much lower pressures and is simpler in design.

Resonance was also a problem in the development of the cryogenic engine of the US space shuttle, according to Tadeo Arimoto of NASDA's international affairs division, and NASDA is seeking information from the US National Aeronautics and Space Administration (NASA) to try to solve the problem, which, according to Arimoto, may require thickening and redesign of the turbine blades.

The first test launch of the H-II originally scheduled for early 1992 has been postponed until 1993, and this will cause delays in the subsequent launch of NASDA satellites. For example, the Space Flyer Unit, a multi-use platform being developed by NASDA, the Ministry of International Trade and Industry and the Institute of Space and Astronautical Sciences, will now be launched along with a geostationary meteorological satellite (GMS-5) in early 1994 instead of early 1993. And the launch of the Advanced Earth Observing Satellite (ADEOS), which at one time was tentatively scheduled for 1993 (later changed to 1994), is now postponed until early 1995.

David Swinbanks

French haemophiliacs awarded damages

Paris

FRENCH haemophiliacs infected with the AIDS virus through transfusions of contaminated blood products will begin receiving compensation from September this year. Systematic screening of blood products was introduced in France on 1 August 1985 but by then almost 1,500 of the 3,500 haemophilia sufferers had been infected. Of this number, about 200 have developed AIDS and 80 have died.

A two-tier trust fund has now been set up by the Ministry of Health and Social Security and private health insurance companies. The state fund is to be financed through the new government association for the campaign against AIDS and will be reserved for confirmed AIDS sufferers. These will receive between FF30,000 (\$4,800) and FF170,000 (\$27,200) accord-

ing to their personal and family circumstances. Private health insurance companies, which will receive tax benefits partially to offset the burden of the compensations, will make payments to seropositive haemophiliacs (FF100,000) and their seropositive partners, if these were contaminated through sexual relations (FF100,000). The family of a haemophiliac who has died of AIDS will be eligible for compensation of up to FF225,000 (\$36,000).

The fund will be managed by the Fondation de France in collaboration with the French haemophilia association. The awards are unusual in that state responsibility, through the national blood transfusion service, has not been proven in the courts. A condition of the awards, however, is that individuals agree not to take legal action.

Peter Coles

Japan's modest step forward

Tokyo

JAPAN last week took a tiny step towards supporting research in foreign countries. The New Energy and Industrial Technology Development Organization (NEDO), a semi-governmental agency supervised by the Ministry of International Trade and Industry (MITI), announced the award of four grants to international teams of scientists based both in Japan and overseas to carry out research on new materials. The grants are part of a small but growing effort by Japan to contribute to international scientific research.

NEDO, originally established to develop new energy sources following the oil crisis of the 1970s, was re-organized last year to take over many of MITI's major research and development projects including some budding international programmes (see *Nature* 333, 4; 1988). Earlier this year NEDO awarded a handful of grants for a 'pilot' Human Frontiers Program — Japan's much talked about programme for international research on the brain and molecular recognition. The conditions for the material science grants are very similar to those of Frontiers.

Research teams must be composed of scientists based in two or more countries one of which must be Japan. The grants are worth ¥30 million (about \$210,000) a

year and can be extended up to a maximum of three years.

Last week's four awards go to researchers in Japan, the United States, Britain, Italy, West Germany and Korea. NEDO officials hope to expand their grant programme next year to include research on the global environment, but it is not yet clear if NEDO will be involved in MITI's ambitious plans to boost environmental research in Japan. A decision is expected to be reached by the end of next month, when budget proposals for fiscal year 1990 are to be submitted to the Ministry of Finance.

Apart from the small pilot Frontiers and material research programmes, NEDO is in charge of several new large research projects which, in theory, are also open to foreign researchers and companies. An example is a marine biotechnology project begun last October into which MITI hopes to pour over a hundred million dollars over ten years with a similar amount from private industry (see *Nature* 333, 4; 1988). Foreign researchers and companies can apply to join the project, but all literature describing it is in Japanese and, according to NEDO officials, as a matter of deliberate policy MITI and NEDO are not advertising such projects in English.

David Swinbanks

EMBRYO RESEARCH

West German ban on embryo research

Munich

A HARD-fought agreement between conservative Bavarians and the rest of West Germany's governing coalition has finally cleared the way for a government ban on research on human embryos. A draft law banning embryo research was approved by the cabinet this week.

It has been clear since the draft was introduced in April 1986 that West Germany would ban some embryo research, especially if it involved germ-line manipulation, the cloning of human beings or the creation of human-animal chimaeras. But science organizations such as the Deutsche Forschungsgemeinschaft (DFG) and the Max Planck Society (MPS) had objected to a total ban on embryo research and the introduction of criminal penalties for violations (see *Nature* 333, 791; 1988). Their plea for limited, strictly regulated exceptions to the law where life-saving therapies might emerge from embryo research seems to have fallen on deaf ears.

The law was stalled for a year because of objections by the conservative Bavarian government. The Bonn government had intended to include a paragraph regulating heterologous insemination, artificial insemination with sperm from someone

other than the husband. For example, the government wanted to forbid a husband who gave his consent to heterologous insemination from later suing his wife for adultery. But Bavaria had objected to allowing any heterologous insemination at all. In a compromise reached last week, the paragraph on heterologous insemination will be removed entirely from the draft law.

In addition to the restrictions mentioned above, the law will ban all use of totipotent cells for research or diagnostic purposes, the use of diagnostic tests to select for the sex of the embryos, and the creation of more embryos than are directly required for *in vitro* fertilization procedures. The law also bans surrogate motherhood, effectively forbidden in West Germany since 1987 through the application of adoption laws.

In order to take effect, the law must still pass both houses of the West German Parliament by next year. Despite their earlier stance, DFG and MPS do not plan any concerted effort to oppose the law. Said one MPS official, the justification for the law is based on "human dignity. If the government wants to forbid [embryo research], we have to accept that."

Steven Dickman

Irvine ordinance

San Francisco

EXPRESSING disgust with slow-moving national and international environmental policies — and firmly entrenching itself as a liberal haven in ultra-conservative Orange County — the city of Irvine, California, last week passed a comprehensive ordinance banning the manufacture, sale or distribution of any products containing ozone-depleting compounds (ODCs).

The city calls the local law — which extends to chlorofluorocarbons (CFCs), halon and chemical compounds such as carbon tetrachloride and methyl chloroform often used in cleansing and degreasing solvents — the most comprehensive measure of its kind in the world. "We are really trying to be leaders and set an example for the rest of the nation", says city council aide Katherine Lyon. "The federal and state governments and the international agreements have been too little, too late. If we want to get anything done, we have to start somewhere. . . ."

Irvine is a planned community of 105,000 residents about 50 miles south-east of Los Angeles — and has become home to a variety of electronic, aerospace and other high-technology companies. City officials say the measure, which passed by a 4-1 vote and which will take effect on 1 July 1990, will affect approximately 10 per cent of Irvine's 5,000 businesses.

Supermarkets will have to remove polystyrene containers made with CFCs. And, although coolants such as freon can be used in air conditioners and refrigeration units until an acceptable alternative is available, repair shops must install equipment to recycle the compounds.

The ordinance provides temporary exemption for businesses having trouble meeting the one-year deadline. Also exempted are university research programmes, and medical and military companies that must employ ODCs to meet government regulations.

The ordinance was opposed by some business-people, who argued that singling out one small community placed an undue economic burden on local employers. But Lyon says the new law has widespread support — and that the city has received many unsolicited letters of approval.

This is not the first innovative and controversial programme instituted under Irvine mayor Larry Agran. About a year ago, the city established a voluntary kerbside recycling programme now used as a model around the country. And Irvine has a comprehensive human-rights ordinance which prohibits discrimination on the basis of race, sex, sexual orientation, religion or ethnic origins.

Such actions stand out in staunchly conservative Orange County — and Lyon says several people have termed Irvine the "Berkeley of the south". Robert Buder

Byelorussia collects dose

London

ZIANON Pazniak, chairman of Adradzennie, the Byelorussian Popular Front for *Perestroika*, has accused the Moscow government of artificially producing the rain that in early May 1986 deposited radioactive pollution from Chernobyl on the province of Mogilev. In a "Statement to Western Journalists", Pazniak notes that, after the accident at Chernobyl, just 5 km across the Byelorussian-Ukrainian frontier, the prevailing winds meant that "tonnes of radioactive materials" were deposited in southern and eastern Byelorussia. Then, he said, a few days later, when the wind changed and "the strontium-caesium clouds were moving in the direction of Moscow", rain was artificially induced over eastern Byelorussia to save the Soviet capital.

Not only was this fact concealed from the Byelorussian public and the outside world, Pazniak claims, but no steps were

taken to protect the local population from exposure or to treat those affected.

As a result of the cloud seeding, he says, the radiation level in the south-east of the Mogilev province (between 40 and 100 curie per square km) is higher than in the 30-km zone around Chernobyl, and significantly higher than the 15 curie per square km safety level. More than 100,000 people live in the affected zone, Pazniak notes. Already, 25 per cent of the local children are suffering from thyroid insufficiencies, and the cancer rate is rising. Yet no special medical services have been provided nor has any provision been made by the state for the affected children to go to holiday camps outside the contaminated zone.

The high radiation levels in Mogilev were first revealed last February, with the publication of contamination maps in the Byelorussian press (see *Nature* 337, 683; 1988). In May, further maps were issued, ostensibly as guides to mushroom pickers, which showed a far greater extent of contamination. Adradzennie, Pazniak says, is concerned about the fate of the children from the Mogilev area—but the authorities do not recognize this organization and persecute its representatives.

Unlike similar 'popular fronts' in other Soviet republics, Adradzennie was not even allowed to hold its inaugural congress in its own republic, but had to meet across the frontier, in Latvia. As a result, Byelorussian ethnic activists have been virtually driven into the arms of the far more militant Balts—which, according to leaked confidential party briefing documents, was precisely what the Party authorities hoped to avoid.

Pazniak, 18 months ago an obscure archaeologist employed at the Institute of History of the Byelorussian Academy of Sciences, hit the headlines in June 1988, when he published his account of the excavation of the mass graves of Stalin's purges in Kurapaty woods, on the outskirts of Minsk. Since then, he has become the charismatic figure of the Byelorussian ethnic renaissance, a role confirmed, last month, by his election as chairman of Adradzennie. A few days after the Adradzennie inaugural meeting, a new Byelorussian Ecological Union was established, with the high-level backing of the Byelorussian Peace Committee and other Byelorussian organizations. One of the prime movers in setting up the new organization was Ms S. Rudneva, deputy head of the Department of Nature Conservation of the Council of Ministers of the USSR. But in her address to the founding meeting, she scolded the delegates for demanding action for the victims of Chernobyl. The Chernobyl disaster, she said, had nothing to do with ecology.

Vera Rich

Germans weigh plans

Munich

AFTER years of rhetoric, West Germany has announced a new initiative in research on the Earth's atmosphere. The most significant step could be the launch of a satellite to determine levels of stratospheric ozone and other trace gases.

Always among the first to champion environmental causes at political gatherings such as this month's Paris summit, West Germany has, apart from a few research groups, lagged behind the United States in gathering atmospheric data on which new environmental policies could be based. Other European countries have done equally little, but none has been as vocal in its concern for the environment as West Germany, where politicians have been thinking Green for half a decade.

If built, the proposed satellite (UFS in German) would be the first European satellite devoted specifically to climate change and the environment. UFS, which would have to be launched by 1995 for maximum effectiveness, could help fill a perceived gap in the gathering of data about the ozone layer and the greenhouse effect. Most European satellites so far have been for telecommunications or weather prediction; scientific uses have mostly been limited to extraterrestrial science. France is expected in the coming months to propose a similar environment-oriented satellite.

The UFS feasibility study signals a shift in policy, with West Germany expected to urge the European Space Agency (ESA) to give climate and environmental research a higher priority. ESA support would seem to be a necessity, as costs could reach several hundred million Deutsche marks (1 DM equals \$0.52).

The study will be completed by the end of 1989, and an outline could then be submitted early next year to ESA. A decision must follow quickly if UFS is to be launched before the polar platform, an ESA project, set for launch in 1997 or 1998, whose tasks also include measuring ozone and other trace gases.

West Germany is expected to push for UFS at the same time as it recommends a delay of the ESA satellite ERS-2 (Earth Resources Satellite-2). There has been talk of including ozone measurement equipment on ERS-2, but this would be superfluous if the launch date is pushed back to 1997, as the West Germans are urging.

UFS would measure ozone, nitrous oxide, methane and other trace gases, and would include oceanographic instruments to assess global warming and other climatic trends. US satellites have done the bulk of these measurements so far, but one ozone detector aboard the NIMBUS-7 weather satellite has already exceeded its planned life-span and a replacement, the Upper Atmosphere Research Satellite, is not expected to be launched until 1992.

Steven Dickman

NUCLEAR PLANTS

Damages for residents

Boston

IN A settlement marking the first time the US Department of Energy (DoE) has conceded local damage from a nuclear weapons plant, the agency has agreed to pay \$73 million in damage claims brought by 14,000 residents living near its Feed Materials Production Center in Fernald, Ohio.

The money is intended to compensate Fernald residents for emotional distress and for significant drops in property values due to their proximity to the nuclear weapons plant. A portion of the money will also be used to fund epidemiological studies of the health of people living within five miles of the plant's perimeter.

The Fernald plant, which processes uranium for the rest of the nation's nuclear-weapons complex, also ranks in volume as the third largest radioactive waste dump in the United States, behind Hanford in Washington state and the Savannah River plant in South Carolina. But in certain categories, the Fernald plant's problems are worse: the US Environmental Protection Agency has declared the Fernald plant the nation's biggest emitter of uranium.

During the trial, DoE maintained repeatedly that uranium releases from the Fernald facility presented no danger to local residents.

After the settlement was announced, DoE attorney Henry A. Gill said the decision reflected only a sense of "uncertainty" about the environmental health effects of the emissions, not an acknowledgement that emissions led to physical illness.

Seth Shulman

A shadow over immunoassay

Roger Ekins

The technique of immunoassay is widely used in all branches of medicine. Yet an ugly patent dispute bodes ill for its future availability.

In 1975, Milstein and Köhler published their pioneering studies on the *in vitro* synthesis of monoclonal antibodies¹, for which they were awarded the Nobel prize in medicine in 1984. The use of such antibodies for immunoassays, with an estimated annual market value in excess of \$5,000 million², was the subject of patent applications filed in 1980 by several companies in the United States and Europe, and has since become the focus of increasingly bitter patent disputes. The most widely publicized is the prolonged legal battle in the United States between Hybritech Inc. (a subsidiary of Eli Lilly), Monoclonal Antibodies Inc. and, more recently, Abbott Laboratories Inc. over the Hybritech patent covering the use of monoclonal antibodies in 'sandwich immunoassays'^{3,4}. The conflict is likely to spread to Europe and elsewhere as similar patent applications are reviewed, and the full implications of their enforcement are perceived.

Legal processes

Litigation between Hybritech and Monoclonal Antibodies began in 1984, and was initially highlighted by a 15-day hearing in August 1985 before the district court for the northern district of California. The court's finding, based on US statutes relating to novelty, obviousness and enablement, was that the Hybritech 'sandwich assay' patent (filed on 4 August 1980) was invalid. 'Obviousness' is a difficult judgement based on comparison of what was known beforehand with what the patent discloses. 'Enablement' requires that the patent application reveals to those skilled in the field how to practise the patented invention. Hybritech appealed against the decision, and in September 1986, the court of appeals for the federal circuit reversed the decision, finding as erroneous the district court's conclusion that use of monoclonal antibodies in sandwich assays was obvious, and finding its decision on the issue of enablement to be "utterly baseless".

Hybritech immediately renewed attacks both on Monoclonal Antibodies and on Abbott Laboratories, demanding (in the latter case) not only damages and a permanent injunction preventing Abbott's sale of monoclonal antibody-based sandwich assays, but a preliminary injunction preventing Abbott's distribution of such products before the case came to trial⁵. In 1987, the court issued preliminary injunctions first against Monoclonal Antibodies⁴ and, later, Abbott²,

and these have subsequently been confirmed. Although this long-running saga has yet to be concluded, the Hybritech patent remains firmly in place, and companies in the United States must either apply for a patent licence (which Hybritech can refuse), or risk the possibility of being judged guilty of wilful infringement, for which offence damages are tripled.

In Europe, patents relating to closely similar 'inventions' were filed on 9 April 1980 by the UK National Research and Development Corporation (a sandwich assay for hepatitis B surface antigen based on monoclonal antibodies); on 25 April 1980 by Hoffman La Roche (for a sandwich enzyme-immunoassay); on 20 June 1980 by Unilever (the formation of sandwich complexes between antigen and dual monoclonal antibodies); and on 28 July 1980 by Organon (also describing such complexes). Hybritech also filed in several European countries, the effective filing

date being 4 August 1980. The validity of these patents, however, has not yet been tested in the courts.

Ramifications

Although the existence of the patents is clearly of critical importance to the manufacturers of immunodiagnostics, it is also of concern to universities, health authorities and other institutions which distribute immunoassay reagents or rely on the use of these techniques in research and diagnostic activities. Use by any individual or organization of a patented discovery in a way causing loss of profit to the patent holder exposes the user to claims for compensation. A shadow thus hangs over the development of improved methods for the routine screening of blood for viral antigens or antibodies (for example, those relating to hepatitis and HIV) or hormone deficiency (such as neonatal hypothyroidism).

The legal impediments to the proper exploitation of Milstein and Köhler's discoveries are inevitably causing considerable disquiet throughout the scientific community. Concern also derives from the highly questionable grounds on which patents in this area have been granted. Incredulity is repeatedly expressed that what was seen in the late 1970s as an obvious area of application of Milstein and Köhler's techniques, and the subject of open discussion, publication⁶, international teaching and publicly disclosed research, should have been effectively hijacked by manufacturers using scientific arguments widely regarded as misleading and specious. Such events throw into sharp relief the doubts increasingly felt regarding the ability of existing legal institutions to discriminate — in areas of increasing scientific complexity — between spurious claims to novelty and genuine invention.

To comprehend the significance of the Hybritech/Abbott dispute, both the importance of immunoassay in medicine and industry, and the special advantages of the 'two-site' approach, must be appreciated. Immunoassay techniques are now used for the sensitive measurement of biologically important substances and are unlikely to be supplanted in the foreseeable future. Their ubiquity derives largely from the unique ability of an individual antibody to distinguish molecules of a particular structure, enabling appropriately selected antibodies to be used to measure trace amounts of many different substances in blood and other complex

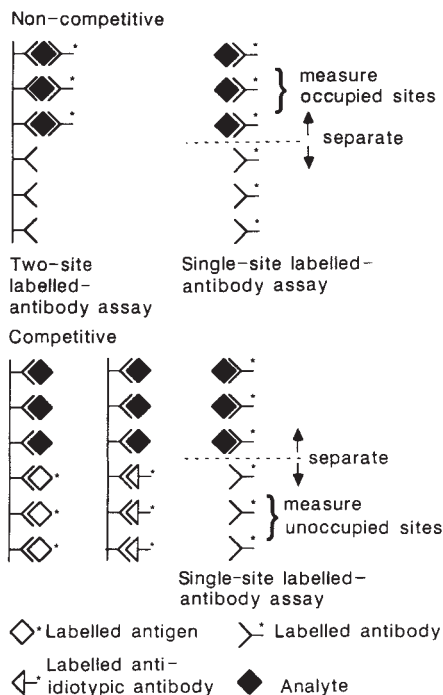


FIG. 1 Alternative methods of measurement of antibody binding site occupancy. Labelled antibody methods can be of competitive or non-competitive design, depending on which fraction (analyte-bound or unbound) is measured. Reactants may be introduced in any order, although assay sensitivity may thereby be affected, and optimal reactant concentrations may differ.

biological mixtures. Immunoassay methods are now used for cancer detection, monitoring of drug therapy, pregnancy and allergy testing, and as diagnostic tests in endocrinology, haematology and many other disciplines. They are also used in key areas of medical research; for example, in studies of human reproduction and the operation of the central nervous system. Immunoassays are increasingly used in other biologically related fields; for example in the food industry (for the detection of contaminants or storage deterioration), in the pharmaceutical industry, in agriculture, in forensic investigation and in environmental research. Not surprisingly, the manufacture of immunoassay kits and related instrumentation now constitutes the world's largest biotechnology-based industry.

Methodology

In the 1970s, most of these kits were of 'competitive' design, a notable exception being the Abbott kit for hepatitis B surface antigen. Conventional competitive assays are generally of the radioimmunoassay (RIA) type, involving a radioisotope-labelled antigen, whose purpose is to reflect antibody binding to the unlabelled antigen (the analyte) in the test sample. The key feature of a competitive assay is that maximal assay sensitivity is attained using an amount of antibody tending to zero. An alternative to using labelled antigen is to label the antibody itself^{7,8}, yielding — if the label is a radioisotope — an immunoradiometric assay (IRMA). Like labelled-antigen methods, certain labelled-antibody assays (those in which the signal from antibody not bound to analyte is measured) necessitate use of an antibody concentration approaching zero, and may similarly be termed competitive. However, methods in which the signal from labelled antibody bound to analyte is measured uniquely require an optimal amount of antibody tending to infinity⁹, and are termed non-competitive (Fig. 1).

Competitive and non-competitive immunoassays differ significantly in performance characteristics as a result of the differences in optimal antibody concentrations on which they rely. Most particularly, they differ in their potential sensitivities (Fig. 2). Much higher sensitivity is attainable using the non-competitive approach, particularly if the label used is of higher specific activity than the radioisotope ¹²⁵I commonly used in this context¹⁰. Whereas the lowest analyte concentration detectable using a competitive design is of the order of 10⁷ molecules per millilitre, a non-competitive method is potentially capable of measuring concentrations lower by several orders of magnitude¹¹. Non-competitive designs also give a higher reaction rate between analyte and antibody, so far shorter incubation times are possible.

Nevertheless, a price attaches to these benefits; this derives from the tendency of a large amount of antibody to bind molecules differing from, but with structural resemblance to, the analyte itself, implying a loss of assay specificity. This can be overcome by preliminary immunoextraction of analyte molecules using a second antibody (usually directed against a different binding site, or epitope). This technique of sandwich, or two-site, immunoassay¹² combines the virtues of ultra-high sensitivity, specificity and short reaction time, features of crucial importance in many biomedical applications. Despite these advantages, penetration of sandwich methods into the immunoassay-kit market was initially slow. The principal impediment was the difficulty of repeatedly preparing large amounts of purified labelled antibody from conventionally produced antisera. Purification techniques are intrinsically tedious and wasteful of antiserum, itself often costly to produce. This fact, coupled with the much higher amounts of antibody demanded by non-competitive methods, dissuaded many manufacturers from developing kits of this kind.

Advance

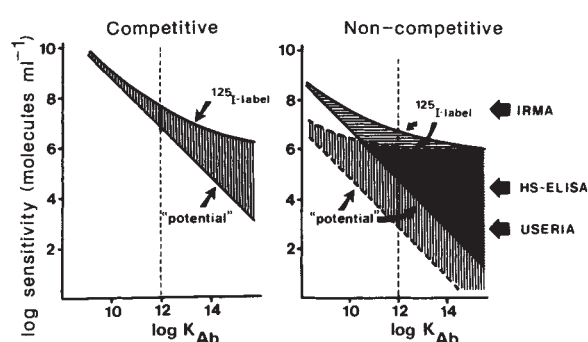
The methods of *in vitro* monoclonal antibody production developed by Milstein and Köhler constituted a major breakthrough, permitting in principle the synthesis of infinite amounts — in relatively pure form — of a single species of antibody directed against a single antigenic site. The applicability to immunoassay of antibodies prepared in this way was immediately obvious. Though there are clearly no *a priori* reasons why monoclonal antibodies should not be used in any type of immunoassay, it was clear that the advantages of Milstein and Köhler's techniques related particularly to sandwich methods.

There is evidence that this concept was perceived by research groups throughout the world as soon as Milstein and Köhler's

findings were announced. For example, following discussions in 1976 with Milstein and the WHO, I. Roitt (professor of immunology at the Middlesex Hospital Medical School, London) and I submitted a grant application (approved in 1977) for the development of monoclonal-antibody-based sandwich and competitive hormone assays for use in the WHO human reproduction programme. My colleagues and I made no attempt to maintain confidentiality; indeed I drew attention to the potential importance of the hybridoma techniques of antibody production (particularly as applied to sandwich immunoassay)^{5,6}. Likewise, N. Hales, pioneer of labelled-antibody sandwich-assay methodology, began similar studies (also with Milstein's assistance) at Cambridge in 1978.

Although work along identical lines by several research groups is indicative of an idea's 'obviousness', considerable delay in the attainment of a widely recognized objective might suggest that particular problems attach to it which require inventive steps for their solution. Why, it may be asked, did it take so long for assays based on monoclonal antibodies to be developed? Apart from the logistical difficulties many smaller groups encountered in establishing appropriate laboratory facilities, the identification of an antibody with the affinity and specificity characteristics required for the assay of an analyte at the concentration at which it exists in a particular fluid is often arduous and time-consuming, luck playing a large part. Moreover, whether a conventional or hybridoma-based antibody production method is used, the time required to produce a suitable antibody varies considerably from one analyte (or one area of application) to another. Much more stringent sensitivity and specificity requirements must be fulfilled by antibodies suitable for the immunoassay of, for example, free thyroxine or parathyroid hormone in human serum than for the measurement of chorionic gonadotrophin in urine (the basis of the 'home' pregnancy

FIG. 2 Predicted assay sensitivities (molecules per ml) plotted against antibody affinity (L/M). "Potential sensitivity" curves assume a label of infinite specific activity, implying zero error in label measurement *per se*. For competitive assays, other errors are assumed to cause a 1% c.v. in assay response. For "non-competitive" assays, nonspecific binding of labelled antibody of 1% (upper curves) and 0.01% (lower curves) is assumed. Sensitivities using ¹²⁵I are also plotted, sensitivity loss being due to "counting" errors. In practice, highest antibody affinity constants normally approximate 10¹² L/M, albeit antibody affinities seldom exceed 10¹¹ L/M. Higher sensitivities are attainable using monoclonals in a non-competitive mode than by using higher affinity antibodies in a competitive assay. Greater sensitivities require labels of higher specific activity than ¹²⁵I. Sensitivity of non-competitive assays using ¹²⁵I (IRMA), and enzymes (yielding fluorescent (HS-ELISA) or radioactive products (USERIA)) are shown.



tests now widely available). Development of a reliable immunoassay also entails far more than the identification of antibodies reactive with the chosen analyte: it requires extensive investigation of the sensitivity, precision and specificity of the system, so it may take months or years before a reliable, adequately sensitive, assay for a particular substance can be legitimately claimed to have been established. This task — arduous enough even when conventional methods of antibody production are used — may take even longer when *in vitro* techniques are used. Nevertheless, once an adequate hybrid cell line is identified, the benefits of long-term and stable production of a pure 'immortalized' antibody generally justify the initial investment required. None of the techniques involved differs from those originally described by Milstein and Köhler, and none of the companies which ultimately applied for patents in this area disclosed inventive or 'enabling' steps in the methods used for antibody selection. All, indeed, that was required to isolate suitable antibody-producing cell lines was time, care, perseverance and good fortune. Not surprisingly, the time which different groups took to develop monoclonal antibody-based immunoassays differed, depending largely on the particular analyte(s) on which they focused.

Novelty

What claims to inventiveness or novelty have therefore been advanced by companies justifying their patenting (in 1980) the use of monoclonal antibodies in immunoassays? As no genuine basis for such claims exists, it is not surprising that the companies involved have experienced some embarrassment on this point. The most ingenious has been Hybritech, whose researchers contrived to give some superficial appearance of discovery to their experiments by emphasizing the 'unexpected' finding that equilibrium was reached more rapidly in a sandwich system relying on monoclonal antibodies than in one relying on conventionally produced antibodies. This observation formed the basis of the claim that sandwich assays using monoclonals are performed in a shorter time and are more sensitive. These claims, endowing monoclonals with apparently unique, superior and unanticipated properties, have no validity or scientific basis and are totally rejected by academic specialists. They disregard, for example, the fact that the rate of a reaction between two or more reactants depends on the concentrations of the reactants in the system; thus an observation that one reaction proceeds more rapidly than another reveals nothing about the relative kinetic properties of the reactants themselves. There is thus no truth in the suggestion that monoclonal antibodies react more rapidly than those

produced conventionally. Similarly, there is no valid evidence to support the claim that the monoclonal antibody-based system described in the patent was of higher sensitivity than the one relying on conventionally produced antibodies with which it was compared. The sensitivity of an assay system is represented by its lower limit of detection, but no data relating to this quantity are revealed in the patent. Even if the monoclonal-based assay had been shown to be of higher sensitivity, this would have been a fortuitous finding, simply reflecting the relative physicochemical attributes of two individual pairs of antibodies, and unrelated to methods of production.

Predictability

Scrutiny of the Hybritech patent thus reveals nothing that is novel or which was not obvious, well known and publicly discussed before the patent was filed. It reveals neither new concepts nor technical developments. Its suggestion that monoclonals possess unexpected physicochemical properties distinguishing them from antibodies produced by conventional means is specious. The claims it contains regarding the supposed superiority of monoclonal antibody-based immunoassay systems are unsubstantiated by any genuine experimental evidence. Judge Conti, presiding over the district court of the northern district of California, concluded after the August 1985 hearing that the Hybritech patent was invalid on the grounds that "once the scientific community had the monoclonal antibody, it was obvious and logical to those experts in the field to use them in known assays as substitutes for products (polyclonal antibodies) of inferior qualities". The only criticism that can be made of this entirely accurate assessment of the situation since 1975 is that monoclonals produced by cell hybridization techniques are not generally superior to those produced by normal methods; only the means by which a monospecific antibody is isolated and produced — the discovery of Milstein and Köhler — offers advantages. Conti's judgement was nevertheless overturned by the court of appeals of the federal circuit in September 1986. On the issue of obviousness, the federal circuit court stated that "objective evidence such as commercial success, failure of others, long-felt need and unexpected results must be considered before a conclusion on obviousness is reached", and, on these grounds,

rejected Conti's original conclusion. Thus the matter rests.

Whatever conclusions are ultimately reached by the courts, the simple truth is that the desirability of, and reasons for, exploiting Milstein and Köhler's discovery to produce antibodies for immunoassay purposes were not only obvious from the time of their original announcement, but were the subject of international teaching long before any relevant patents were applied for. Monoclonals produced by these techniques display no unexpected features making them intrinsically different from those produced by conventional isolation procedures. Moreover Hybritech's 'inventors' introduced neither novel concepts nor inventive steps, nor did they genuinely demonstrate any unexpected qualities of antibodies produced in this way. Nor indeed did they produce any data of the kind normally required in this field demonstrating their successful development of an accurate, reliable immunoassay using such antibodies.

It is to be profoundly hoped that these facts, widely understood by those in the field, will also be recognized by the courts. For the reality of the present situation is that, by successfully patenting the use of antibodies produced by Milstein and Köhler's techniques, commercial companies have succeeded in implicitly patenting the invention itself. This is a subterfuge too important in its long-term implications to be the subject of decisions by courts unfamiliar with the scientific issues involved. Though some comfort may be drawn from the fact that final rulings on the patents' validity have not yet been made, costly legal disputes between large companies are, in practice, often resolved by private agreements between them, thus retaining important patents in place to their mutual benefit. Maintenance of the monoclonal antibody patents thus represents a possible outcome of the present disputes. The resultant effect on developments in this field is a matter on which the entire scientific community has both the right and the duty to comment, and to make its concerns known. □

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1. Köhler, G. & Milstein, C. *Nature* **256**, 495 (1975).
2. Ezzell, C. *Nature* **327**, 5 (1987).
3. Ezzell, C. *Nature* **324**, 506 (1986).
4. Ezzell, C. *Nature* **326**, 532 (1987).
5. Ekins, R.P. in *Radiopharmaceuticals II: Proceedings, 2nd International Symposium on Radiopharmaceuticals* 219 (Seattle, Washington, March 1979).
6. Ekins, R.P. *Nature* **284**, 14 (1980).
7. Wide, L., Bennich H. & Johansson S.G.O. *Lancet* **II**, 1105 (1967).
8. Miles, L.E.M. & Hales, C.N. *Nature* **219**, 186 (1968).

9. Jackson, T.M., Marshall, N.J. & Ekins, R.P. In *Immunoassays for Clinical Chemistry* (eds Hunter, W.M. & Corrie, J.E.T.) 557 (Churchill Livingstone, Edinburgh, 1983).
10. Dakubu, S., Ekins, R., Jackson, T. & Marshall, N.J. in *Practical Immunoassay. The State of the Art* (ed. Butt, W.R.) 71 (Dekker, New York, 1984).
11. Ekins, R.P. in *Radioimmunoassay and Related Procedures in Medicine* 241 (IAEA, Vienna, 1978).
12. Wide, L. in *Radioimmunoassay Methods* (eds Kirkham, K.E. & Hunter, W.M.) 405 (Churchill Livingstone, Edinburgh, 1971).

Women's rights at 'Einstein'

SIR—Leonard Minsky and Rita I. Kaplan (*Nature* 339, 10; 1989) suggest that there is a contradiction between my opinion on the specific case of Sobel vs. Yeshiva University, and the sentiments of the letter on the same page written by myself and nine other women faculty members of the Albert Einstein College of Medicine in response to Joseph Palca's unsubstantiated statement (*Nature* 338, 192; 1989) that "Einstein has had a poor track record in its attitude towards female faculty".

My position, reflected in my published remarks to the senate as chairperson of the senate committee, was to urge the Yeshiva administration to settle the case without further costly litigation. This was formalized in a unanimous resolution by the senate to that effect. Some of my remarks are quoted out of context, and convey a false impression that they arose from my "anger with the continuing pattern of discrimination" at Einstein. Rather, the words expressed my disappointment at the handling of the senate resolution by the administration, and the failure of the administration to undertake steps to settle the case promptly.

The Sobel case illustrates the difficulties at Einstein and other US medical schools that stemmed from, and reflected, the then current and historical national attitudes on issues of employment and salaries for women professionals. In 1972, a few years before the class suit was filed, the women's committee of the Einstein faculty-student senate made a comparative study of employment of women at Einstein and other medical schools (how many academic senates had women's committees then?). I was chairperson of that committee. While pointing out that much had to be done to rectify the differential salaries for women, and making specific recommendations on recruitment, promotions, salary adjustments, etc., the committee found that Einstein had more women on its faculty, and more women as students, than most other medical schools. In the ensuing years Einstein, pressed by a vigilant and forward-looking faculty, moved to rectify the deficiencies pointed out in the report. Even today, after a decade of increasing opportunities for women in academic medicine, Einstein leads the major medical schools in the numbers of female faculty and students (based on data from the Association of American Medical Colleges and New York State Education Department). At Einstein, women are recruited (at the same salary levels) and promoted to senior rank under the same criteria as those governing recruitment and promotion of men. That is why Einstein leads the other medical schools in numbers of women in the senior ranks of

associate and full professors.

Most importantly, the faculty here is fully united in regard to women's issues and is constantly vigilant concerning wrong-doing to any faculty member whether male or female. In this imperfect world we are not always successful in monitoring and correcting inequities, but we persist; hence my remarks to the Senate quoted in the two letters.

Finally, I want to urge Mr Minsky and Ms Kaplan not to make the mistake of thinking that women such as I are automatically weak and incapable of independent and spontaneous opinion.

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Wrong number

SIR—I agree wholeheartedly with your argument for the autonomy and academic freedom of institutions (*Nature* 333, 445; 1989), but I have to differ with you on a small question of numbers. There are 29 designated polytechnics in England and one in Wales. The English polytechnics, along with 55 colleges of higher education, are now independent corporations, largely funded through the PCFC (Polytechnics and Colleges Funding Council). In addition, two Scottish central institutions have adopted the term 'polytechnic', namely Napier Polytechnic of Edinburgh and Glasgow College, which styles itself 'a Scottish Polytechnic'.

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Scientific fraud

SIR—I believe it is unlikely that the professional perpetrators of scientific fraud are mentally ill, or seek self-destruction (*Nature* 339, 91; 1989). These men are brilliant in scientific research, and as Efraim Racker comments, excellent in other fields, too. Why should such men commit fraud? Perhaps there is a plausible explanation.

The eventual 'professional fraud-maker' is a smart and an intelligent person who has always done brilliantly in school and colleges. He is much aware of his competence, and, used to success as he is, expects instant results and recognition in research too. The firm conviction that he is brilliant, along with the belief that brilliance alone begets success, exerts immense pressure on him to prove himself at everything he attempts; and if the results

do not come soon enough, this desperate smart scientist resorts to 'short cuts'. The perpetrator of fraud is aware that his 'sensational' results will be checked and proved wrong in the course of time, but by then he has capitalized on them. He already enjoys the reputation of being a brilliant scientist and reasons that people will more easily accept his frauds initially, and later the explanations that he either made an honest mistake (brilliant scientists are known to have made silly mistakes!) or was victimized by a jealous colleague. He therefore does not intend to be caught.

Perhaps for this reason, Racker's presumption that his brilliant student was ill and that there was no cure for his illness is unfounded. Scientific fraud is a crime, and like other crimes may emanate from ambiguous values of society. The problem needs to be analysed and possibly solved. Those who do science should not claim that it cannot be.

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Too high a price

SIR—I was pleased to read the favourable mention given to my contribution by Stephen C. Inglis in his review (*Nature* 339, 188; 1989) of *RNA Genetics* (CRC Press, 1989). I was at the same time distressed to learn that hardly anyone else will ever read it, as the outrageous price of the 3-volume set (\$425) puts it out of reach of virtually all individuals and laboratories as well as most libraries. Lest I give the impression that I, as an author, am in a privileged position in this regard, I hasten to point out that the publishers, in their generosity, provided me with only the one 17-mm thick volume containing my own chapter. Nor was the 'honorarium' paid me by the publishers sufficient to purchase even one of the other two volumes. Like nearly everyone else, I will never read most of this potentially useful work.

The moral is clear. Had I been aware of the price of the book, I would never have agreed to contribute to it. In future, I will always find out the pricing and distribution policy of the publisher of any work to which I am asked to contribute, and will deal only with those who make an honest attempt to make the efforts of their workers accessible to the relevant readership. I urge all readers, as prospective authors, to do the same. Perhaps we can make a dent in the overpublication of such excessively expensive and unread books.

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... and then there was none?

SIR—Those who fear for the future of Medical Research Council (MRC) support for science may have had their sensibilities excited by an advertisement for the new post of head of public relations for the council, which appeared in *The Times* of 28 June 1989.

The appointee was "expected to establish close links with the media, Parliament and with the Council's research scientist" (my italics). The singularity implied in this statement has an ominous ring!

Incidentally, university academics with frustrated professorial ambitions might find PR work for the MRC rather more rewarding financially. In addition to the flexible working hours and generous holidays that they already enjoy, an attractive annual salary of up to £30,000 (upon proven merit) is on offer.

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Ozone layer tax

SIR—We are all aware that a whole class of chemicals — chlorofluorocarbons (CFCs) — cannot be used freely as they once were because of their destructive effect on the upper-atmospheric ozone layer. And, to make things worse, carbon tetrachloride and methyl chloroform must be added to the list of ozone-depleting chemicals. Even CFCs that are not fully halogenated (HCFCs) are not totally innocuous in this regard.

Given the consequences of continued ozone-layer depletion, one can understand why concerned people want CFCs and related substances banned altogether. I am writing to argue for another, and more flexible, course of action to protect the ozone shield.

I believe every nation should tax ozone-depleting chemicals, heavily if necessary, at the point of manufacture or importation. The more destructive a given compound, the more heavily it should be taxed.

A tax on ozone-depleting chemicals would have the following benefits. First, those who engineer refrigerators and air conditioners would have a powerful incentive to make their designs leak-free. Second, establishments that repair such appliances would have an incentive to recover and recycle CFCs, rather than let them escape. Similarly, industries that use these chemicals for vapour degreasing would be financially motivated to recover, clean up and use them over and over again. Finally, a tax on chlorine- (or bromine-) containing compounds, in proportion to their ozone-depleting

power, would motivate engineers to use the least harmful compound feasible in a given application.

The idea behind my proposal is to reduce upper-atmospheric ozone depletion as much as we have to, with the least inconvenience to businesses and consumers alike.

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No lysenkoism

SIR—Vera Rich (*Nature* 337, 8; 1989) writes that the changes in the Academy of Sciences in Sofia include the liquidation of lysenkoism in Bulgaria. This is not true. We are not aware of any concrete arguments supporting this conclusion. But it is known that in 1949 there were crude administrative efforts to introduce the theory and personal views of Lysenko in our biological science. Then, in 1956, these administrative efforts were officially discouraged and rejected as unacceptable for the development of science.

Thus Lysenko's influence in Bulgarian scientific investigations was temporary and insignificant. The Bulgarian Academy of Sciences also maintains contacts and encourages scientific collaboration and research with the academies of all the socialist countries and with the leading scientific organizations in France, Italy, West Germany, Spain, Switzerland, Sweden, the United States and other countries, as well as with the Royal Society and the British Academy. These collaborations have resulted in measurable scientific achievements. I am concerned that Vera Rich's article does not contribute to the improvement of positive interactions between scientists, either in the West or East.

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Room for theory?

SIR — V. A. Huszagh and J. P. Infante (*Nature* 338, 109; 1989) ask whether "the hypothesis in the biological sciences [is] an endangered species". They conclude that it is, notwithstanding the value of (for example) Mitchell's chemi-osmotic hypothesis of oxidative phosphorylation.

The authors point out that "those who review hypotheses in biology are usually data-generators who do not know the elements of importance, and who thus make non-scientific sociologically based objections that are irrelevant to the construction or purpose of a hypothesis", adding that "it took six years after he published them for Mitchell's ideas to be

seriously tested by others".

The fact that you published these comments suggests that in principle you agree with them. However, on looking over your Guide to Authors (for example *Nature* 339, 252; 1989) I find that of the six categories of paper listed, none seems to make room for theoretical work.

"Articles are research reports.... Letters to *Nature* are short reports of novel findings.... Commentary articles deal with issues in, or arising from, research.... Review Articles survey recent developments in a field...." And News and Views or Scientific Correspondence rule themselves out as likely places for major or even minor theorizing.

I note that, according to Huszagh and Infante, Mitchell's theoretical paper was published in *Nature* in 1961 (191, 144–148; 1961). In which of the above categories would such a paper fit today?

C.M. FAIR

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■ *Nature* does not necessarily agree with the opinions expressed by the authors of Commentary articles. Mitchell's paper was, of course, more than a mere hypothesis; a model of its kind, it begins with a careful marshalling of evidence for the need for a new hypothesis and a demonstration that the chemi-osmotic hypothesis will account for otherwise inexplicable observations. Editor, *Nature*.

SETI

SIR—R. A. Michaels (*Nature* 339, 500; 1989) suggests that extraterrestrial civilizations might send out distress signals if they were aware of an imminent supernova in their vicinity. That seems to be unlikely, as it is probably not possible to predict such an event with sufficient accuracy. There might, however, be other reasons for concentrating the search for extraterrestrial intelligence (SETI) on the vicinity of recent supernovae.

A civilization wishing to signal its presence would do so in a manner that would optimize the chances of detection. That would mean transmitting in the direction opposite in the sky to any recent supernova, as it would be fairly certain that any civilization along that line of sight would be turning its best optical and radio telescopes in the direction of the supernova, if able to do so, just as we did immediately after 1987A.

The two immediate implications are that we should be conducting SETI work in the general direction of 1987A while ourselves transmitting, if we so desire, in the direction of ω Draconis.

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Quasicrystals stabilized by entropy

Fivefold symmetry in the solid state remains a puzzle, if only because credible realizations are statistically special structures. But now it seems likely that they are not so special.

WHATEVER happened to quasicrystals? More than four years have gone by since it was first reported by Steinhardt and Schechtman and their colleagues (see *Nature* **313**, 263; 1985) that alloys of manganese and aluminium may crystallize with what appears to be fivefold symmetry, as inferred from X-ray and electron diffraction. The development was surprising because crystal lattices with fivefold symmetry are incompatible with the translational symmetry of infinite crystals. Even after five years, it remains unclear why they exist.

There are several schemes for arranging atoms in ways that would give diffraction patterns fivefold symmetry, the simplest of which (in two dimensions) is the Penrose tiling of the plane by copies of two rhombi, the sides of which are equal, but which are respectively thin and fat. There have also been three-dimensional generalizations of the Penrose tiling, involving not merely the predictable icosahedra (platonic solids with natural pentagonal symmetry) but others with names such as tricontrahedra.

The geometrical details of these arrangements are enough to have people reaching for their scratch pads, but the two-dimensional case is sufficiently taxing. If one supposes that each vertex of a rhombus, thin or fat, is occupied by an atom, it is possible to calculate that the diffraction pattern will have fivefold symmetry. The essence of the case is that these arrangements are not crystalline in the sense that it is not possible to shift the pattern in some direction and find that the result coincides with the original, but they are quasi-periodic in the sense that displacements relative to the length of the rhombus side by the 'golden mean' will substantially regenerate the pattern.

Theoreticians have spent much of the past four years looking for neat ways of representing the positions of the vertices in such patterns. The best techniques are those in which a two- (or three-) dimensional pattern is regarded as the projection onto a two-dimensional plane (or a three-dimensional space) of an array of points in a hypothetical lattice with a greater number of dimensions. For example, the Penrose tiling is the intersection of a plane with an array of points in a regular cubic lattice with five dimensions. Other less regular tiling planes would show up when a suitable plane is drawn through a five-dimensional lattice in which there is an appropriate and well-defined array of

points.

Helped by stratagems such as these, many people have spent much of the past four years calculating all kinds of properties of quasicrystals. The exercise has been entertaining for all concerned, but not especially illuminating. Put simply, the Penrose tiling and its analogues show that it is indeed possible to construct arrays of atoms which are infinite in extent, which have no essential defects and which are stable, but nobody is much the wiser for knowing that. The essential difficulty is that these regular structures are highly special structures. Indeed, their construction by projection from, say, a five-dimensional array in which not all the points are occupied onto, say, a two-dimensional slice taken through it shows just that: the original array must be pre-defined, as must the orientation of the slice. Why should arrays of real atoms crystallizing from a high-temperature melt choose to dispose themselves in such a special way when there is no compelling energetic advantage in the arrangement?

That is part of the reason why it has recently seemed that the physics of the quasicrystalline state has been heading for a dead end, but now there are some signs of life. Indeed, two separate groups, apparently independently of each other, have produced calculations of remarkably similar systems comprising atoms of two species, and have been able, by different methods, to arrive at a convincing demonstration that the stability of real quasicrystalline systems arises not because of some decisive energetic advantage but because of the large entropy of these systems. Any specified and ideal quasicrystalline array will be but one of a host of arrays with essentially the same energy, and which are thus degenerate in the technical sense. An apparently essential criterion is that the two species of atoms should differ markedly from each other in size.

The two calculations appear consecutively in the current issue of *Physical Review Letters*. M. Widom and D.P. Deng from the Carnegie-Mellon Institute and C.L. Henley from Boston University (**63**, 310; 1989) and, separately, Katherine J. Strandburg (Argonne National Laboratory), Lei-Han Tang and Marko V. Jaric from Texas A & M (**63**, 314; 1989), start with essentially the same system of a mixture of large and small atoms in a planar array which interact by means of

Lennard-Jones potentials. Given that both articles were apparently received at Brookhaven on 15 September last year, one is tempted wonder whether representatives of the two groups had arranged to despatch their manuscripts from the same airport overnight courier office, although geography may have complicated such an arrangement. Whatever the mechanics, the papers read together have the virtue of filling each other's gaps.

Widom *et al.* state the problem the more clearly. They choose the radii of their atoms (in two dimensions) so that ten small atoms will fit around the circumference of one large atom, and so that five large atoms will fit around one small atom. (Strandburg and her colleagues achieve an essentially similar effect by adjusting the parameters in their Lennard-Jones expressions.) It is then possible to define pairs of thin and fat rhombi on the Penrose model by joining the centres of pairs of contiguous large (or small) atoms with the centres of small (or large) atoms arranged symmetrically on either side of them. Each group then uses five-dimensional space to define the positions of the large and small atoms. "Random tiling" is the name they use to describe what it is doing.

Strandburg and her colleagues carry out a Monte Carlo calculation of such a system which, as elsewhere in solid-state physics, means that they swap structures representing local groups of atoms in such a way as not to change the overall properties of the system, the ratio of large to small atoms for example. More abstractly, Widom and his colleagues set out to calculate the thermodynamic properties of a piece of two-dimensional lattice (wrapped into a cylinder for consistency) by allowing for all possible interactions between one row and its neighbours (which entails, among other things, enumerating all possible configurations of a row).

The outcome is satisfactory agreement. Each group concludes that its model will yield long-range order and sharp diffraction peaks. Widom and his colleagues are the more explicit in their declaration that entropy rather than crude energetics determines the stability of their alloys, but the two groups agree in their estimates of the degree to which this is likely to be the case. Widom and his colleagues modestly add: "It is even conceivable that such a model could apply to real quasicrystalline materials in thermal equilibrium".

John Maddox

Modes of particle transport

Daniel Haft and Michael Edidin

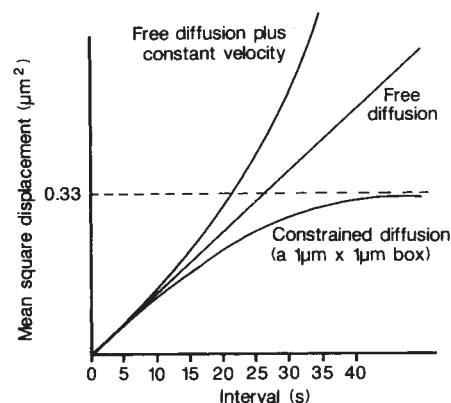
LAST year, Michael Sheetz and colleagues devised a method for looking at small colloidal gold particles in the light microscope and locating them with nanometre precision¹. In a paper on page 284 of this issue², Sheetz and collaborators now report the application of the method to tracking the motion of 40-nm-diameter gold particles attached with the lectin concanavalin A (con A) to the surfaces of living cells. The motion of an individual particle provides a rich harvest of information: in a period of tens of seconds, a particle can switch suddenly and reversibly from a steady, directed transport with little random motion to free diffusion. Analysing the tracks of particles allows Sheetz *et al.* to exclude one proposed model of the control of particle flow through membranes. Furthermore, in an accompanying paper on page 315 of this issue³, Sheetz and colleagues report a previously undescribed phenomenon of particle transport within cells.

The ideal tool for analysing motions in the plane of a cell membrane is a probe small enough to diffuse rapidly, yet bold enough to allow one molecule at a time to be seen. Fluorescent labels can be used to measure diffusion by fluorescence photobleaching and recovery, but this method misses a wealth of detail by reporting only an indirect, bulk measure of diffusion: the rate of change of probe concentration in a micrometre wide region of membrane surface. Variability in the population of molecules in motion and changes in a single molecule's behaviour over time are ignored through averaging. Oversized probes, such as large beads or patches of cross-linked membrane proteins are inevitably caught in the machinery of the cell locomotion and endocytosis. They can demonstrate directed motion, but not diffusion.

The 40-nm gold particle seems to be a probe of near perfect size. Individual particles may be tracked while the molecules they label diffuse freely. A similar approach to that of Sheetz *et al.* tracks single, intensely fluorescent low-density lipoproteins (LDL) over the cell surface by video microscopy⁴. Unlike con-A gold, LDL particles have a well described specificity and valence. However, gold particles can be tracked with greater precision and applied more generally by absorbing ligands other than con-A for other receptor systems.

Sheetz *et al.*¹ bring as much novelty to the analysis of data as they do to its collection. Their analysis settles definitively a long-standing argument about the relevance of directed lipid flow in cell locomotion. Although most evidence suggests that the protein cytoskeleton controls cell

locomotion⁵, the alternative lipid-flow model⁶ has been hard to disprove. In this model, lipid is delivered continually by exocytosis at the leading edge of the cell, sheathing the cell in membrane flowing steadily aft. This flow puts a rearwards force of drag on the structures in the cell that attach to surfaces and so pushes the cell forwards. The same flow is expected to carry all mobile proteins within the cell backwards, except to the extent that endocytosis with recycling or rapid diffusion can oppose the flow. In the model,



Three model cases for diffusion in a membrane (see text).

this current, rather than action of the cytoskeleton, explains the rearwards transport of large surface-bound particles.

To test this hypothesis, Sheetz *et al.* take as their starting point the fact that a sustained current in the membrane would affect the course of a freely diffusing particle, just as a moving sidewalk would bias the random course of a drunkard's walk. However energetic the drunk, the sidewalk's motion would eventually show. If a large enough number of random walks were taken, the average end-position would lie downstream of the start, at a distance fixed by the underlying velocity and the duration of the walk. Sheetz *et al.* watched each of 89 rapidly diffusing gold particles for 20 seconds. Particles from the same microscopic fields, seen in directed transport back toward the nucleus, give the putative direction for rearwards lipid flow. The average rearwards drift is near zero, suggesting that there is no sustained current of lipid flow back from the cell's leading edge. Flow at even a fraction of the speeds seen for steady particle transport would have been detected by the experimenters.

More detail than just the size of the end-to-end jump of a particle can be obtained from a 20-second record of particle position using video microscopy at 30 frames per second. Sheetz *et al.* have developed a theoretical framework to see whether the

assumption of free diffusion breaks down for very long or very short times. A 20-second interval contains within it a number of overlapping 15-second intervals, a larger number of 10-second intervals, and so on, and so the typical size of the jump for a particular size of time intervals can be examined. Better still, the mean-square displacement (MSD) can be determined and plotted against elapsed time. In random diffusion, the MSD should rise linearly with time, and the slope of the plot yields the diffusion coefficient.

Two interesting departures from ideal diffusion can alter the relationship of MSD and time (see figure). One is the presence of an underlying constant velocity. For a particle moving steadily but not diffusing, displacement increases linearly with time, so the MSD should rise with the square of the time — an upward curving parabola with zero initial slope. The rate of change of the slope would reflect the velocity. If a particle diffuses freely and is also subject to a constant underlying velocity, the MSD plot would be a parabola curving upwards from a positive initial slope. The second departure is restriction of free diffusion to a domain small enough for its limits to be encountered in the time-scale of the experiment. The MSD plot would have the initial slope as for free diffusion, but rise with an ever-decreasing slope and approach a horizontal asymptote. The notion that diffusing proteins in cells might lie within domains with impenetrable walls was first proposed for the erythrocyte membrane to explain the free rotational diffusion but hindered lateral diffusion of the membrane-bound protein band 3 (ref. 7). It could also apply to the organization of other cell surfaces⁸.

The relationships of MSD against time reported by Sheetz *et al.* are as expected if lipid flow does not occur. Particles undergoing rapid diffusion rather than directed (cytoskeletal-based) transport have MSD plots that fit best to a straight line, not to a parabola. The MSD plot gives squared displacement, which is a directionless quantity. Unlike the test for average drift, the plot is sensitive even if the underlying velocity from sustained lipid flows is in different directions for different particles. Sheetz *et al.* detected no drift from lipid flow in any direction.

Although the new method allows one proposed mechanism by which cells organize their proteins — the lipid-flow model — to be excluded, it also provides results that complicate our picture of the cell

1. Gelles, J., Schnapp, B.J. & Sheetz, M.P. *Nature* **331**, 450–453 (1988).
2. Sheetz, M.P., Turney, S., Qian, H. & Elson, E.L. *Nature* **340**, 284–288 (1989).
3. Kucik, D.F., Elson, E.L. & Sheetz, M.P. *Nature* **340**, 315–317 (1989).
4. Ghosh, R.N. & Webb, W.W. *Biophys. J.* **55**, 498a (1989).
5. Bray, D. & White, J.G. *Science* **239**, 883–888 (1988).
6. Bretscher, M. *J. Cell Biol.* **106**, 235–237 (1988).
7. Koppel, D.E., Sheetz, M.P. & Schindler, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3576–3580.
8. Yechiel, E. & Edidin, M. *J. Cell Biol.* **105**, 755–760.

surface. In their second paper in this issue³, Sheetz and colleagues describe a new phenomenon of transport: small gold particles bound to the leading lamellipodia of fish keratocytes make occasional forward excursions. Like rearward transport, the motion is dependent on the cytoskeleton. Although the early indications are that this movement is mediated by

myosin, the mechanism for this type of transport awaits interpretation. Application of this new technique to living cells promises to reveal much about cell-surface organization. □

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GEOPHYSICS

Hotspots and mantle convection

Greg Houseman

WILSON's original concept¹ of a hotspot has an elegant simplicity that is perhaps misleading; a plume of hot, partially molten rock punches its way up through the mantle and leaves a trail of volcanic constructs on the surface of the lithospheric plate passing overhead. The Hawaiian–Emperor seamount chain, over 5,000 km long, with ages decreasing monotonically from 80 million years to the present, is the classic example. Details of their relation to mantle circulation are unclear, however. Two new papers^{2,3} cite evidence firstly that thermal plumes are perhaps the only form of thermal upwelling in the mantle, and secondly that the observed spatial distribution of hotspots is entirely consistent with their arising as thermal instabilities in a hot basal boundary layer. But better understanding of mantle plumes still awaits a definitive test of whether mantle convection is primarily single- or double-layered.

Mantle plumes are somehow part of a solid-state mantle convection system which transports about 4×10^{13} W of thermal energy, generated by the decay of radioactive isotopes in the Earth's interior, to the surface. The mantle, about 2,900 km thick (almost half the Earth's radius), is thought by many to behave as a single convecting layer with most of the heat source distributed throughout the layer. An alternative model has two layers with a boundary at about 700 km depth, consistent with geochemical interpretations⁴ of a depleted upper mantle overlying a primitive lower mantle, inside which most of the heat is generated. Since the hotspot concept was introduced, there have been many attempts, using numerical and analogue stimulations and observations of hotspot characteristics, to explain how mantle plumes fit into this poorly understood mantle circulation pattern.

Bercovici, Schubert and Glatzmaier present in *Science*² numerical simulations of thermal convection in a constant-viscosity spherical shell representing the entire mantle as a single layer. In their simulations half or all of the heat input to the mantle is from the core, cylindrical upwelling plumes are the only stable form of active thermal upwelling. In contrast,

cold sinking material takes the form of elongate sheets even though there is no rigid lithospheric layer in the calculation. Similar planforms can be seen in my simulations of a flat layer of convecting fluid with a component of basal heating⁵. In their new study of hotspot locations, Weinstein and Olson³ verify by statistical analysis Morgan's observation⁶ that the spatial distribution of hotspots is strongly biased; they tend to be near mid-ocean ridges and distant from subduction zones. Even allowing for some uncertainty in what is identified as a hotspot, the probability of the observed distribution occurring by chance is less than 1 per cent.

Weinstein and Olson infer from this observation that the distribution of mantle plumes is, if not completely determined, at least strongly influenced by the large-scale convective circulation of the mantle involving the tectonic plates. They interpret hotspots as being due to thermal plumes arising from a basal thermal boundary layer without explicitly identifying the depth of this boundary. The cold subducted plate injected at a subduction zone would spread laterally on the base of the layer and inhibit the nearby formation of hot rising plumes. Their interpretation is also consistent with previously observed inverse correlations of plate velocity with both hotspot density⁷ and kimberlite occurrence⁸.

Thus the numerical simulations indicate that we should expect to see thermal plume structures in the mantle if there is any component of basal heating, and at the same time there is clear observational evidence that hotspots are behaving much as we would expect if they were indeed resulting from the type of thermal plume structure evident in the simulations. The convergence of these two approaches says much for the power of the three-dimensional numerical simulations that are possible with current supercomputers^{2,5}. The evidence clearly supports the view that mantle plumes are essentially thermal in nature, so a significant component of the heat source of the convecting layer is input at its base. But in my view the question of layer depth remains unsettled.

Are we observing plumes that arise

from a thermal boundary layer at approximately 700 km depth with the basal heat source provided by an undepleted lower mantle? Or do the plumes arise at the core–mantle boundary, thereby indicating a significant outflow of heat from the core even though the single mantle layer must be primarily internally heated? The single-layer interpretation seems to imply a significant heat source such as ⁴⁰K present in the liquid-iron core and therefore has important implications for the convection within it and for its thermal and chemical evolution.

Which features of the hotspot distribution are most useful in distinguishing between these two interpretations? One might first consider horizontal spacing. Weinstein and Olson use several hotspot compilations, the largest of which⁹ lists 117 distinct hotspots. If these were uniformly distributed across the globe, the average plume separation at the core–mantle boundary would be roughly 1,100 km, about one-third of the single-layer depth. At the 700 km level the average plume separation would be roughly 1,900 km or nearly three times the upper-layer depth. In fact the hotspot distribution is far from uniform and the nearest-neighbour distance is generally much less than the average separation. If the mantle is single-layered, it is not clear why these thermal plumes are so closely spaced (relative to the layer depth). I am led either to prefer a two-layer model with the observed plumes inhabiting the upper layer or to conclude that not all hotspots arise from separate thermal plumes.

Another promising approach, recently suggested by Davies¹⁰, is to estimate the amount of heat carried by the mantle plumes. He calculates an approximate thermal budget for 26 mantle plumes and concludes that they carry less than 10 per cent of the total heat flow out of the mantle, thus implying an internally heated style of convection and therefore a single-layer model. His estimate of plume strength or magnitude is based on the rate at which new surface topography is generated, and is presumably most accurate for rapidly moving plates such as the Pacific, but is, I think, an underestimate for slow moving plates with anomalously high topography such as the African plate. □

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1. Wilson, J.T. *Phil. Trans. R. Soc. Lond.* **A258**, 145 (1965).
2. Bercovici, D., Schubert, G. & Glatzmaier, G.A. *Science* **244**, 950–955 (1989).
3. Weinstein, S.A. & Olson, P.L. *Geophys. Res. Lett.* **16**, 433–436 (1989).
4. DePaolo, D.J. *Neodymium Isotope Geochemistry* (Springer, Berlin, 1988).
5. Houseman, G. *Nature* **332**, 346–349 (1988).
6. Morgan, W.J. *Nature* **230**, 42–43 (1971).
7. Sahagian, D. *Nature* **287**, 217–218 (1980).
8. England, P. & Houseman, G. *Earth planet. Sci. Lett.* **67**, 109–122 (1984).
9. Vogt, P.R. *J. geophys. Res.* **86**, 950–960 (1981).
10. Davies, G.F. *J. geophys. Res.* **93**, 10467–10480 (1988).

Is imprinting to blame?

Bruce Ponder

THE report by Zhu and colleagues on page 312 of this issue¹, together with that recently published by Dryja *et al.*², suggests that new germ-line mutations to the heritable form of retinoblastoma are more likely to occur on the paternal rather than the maternal chromosome. These authors find no such bias, however, in tumours where the corresponding first mutation has occurred in a somatic cell. This differs from previous reports of Wilms tumour and osteosarcoma^{3,4}, which had indicated a strong preference for the first mutation to occur on the paternal chromosome in sporadic tumours, observations which led to speculation that differences in genomic imprinting of paternal and maternal alleles may be involved^{4,6}. Why the discrepancy, and what do the new data mean?

The background has been reviewed in two recent News and Views articles^{6,7}. Many familial tumours, among them retinoblastoma, osteosarcoma (which arises from mutation at the same locus as retinoblastoma) and Wilms tumour, arise by a 'two-hit' mechanism in which the tumour cells lose first one and then a second allele at a putative 'tumour-suppressor' gene locus. In familial cases, the first mutation is inherited in the germ line. The second mutation occurs in a somatic cell in the target tissue, which then gives rise to the tumour. Histologically similar tumours also commonly occur in a non-heritable form: in these cases, both mutations must occur in the same somatic cell. In either case, the second mutation is often associated with large-scale deletions or chromosomal loss from mitotic recombination or non-disjunction. Because of this, comparison of DNA polymorphisms in normal tissue and tumour from the same individual will often indicate which chromosome has been retained in the tumour and has, by inference, sustained the first mutational event.

Analysis of Wilms tumour and of osteosarcomas that were thought to be of non-heritable type (that is, where the first mutation arose within a somatic cell) showed a strong bias towards retention of the paternal allele. This implies that paternal and maternal alleles must therefore differ in the tissue in which the mutation occurred. The difference might be either in susceptibility to mutation or in expression. If the maternal tumour-suppressor allele was the less active, for example, the descendants of a cell in which the paternal allele had been inactivated as the first event would have relatively little suppressor activity, and would be more likely to develop into a tumour. These differences were thought to be the result of genomic imprinting^{4,6}.

The new data on retinoblastoma^{1,2}, by contrast, show no such bias towards paternal origin of the first mutation in non-heritable tumours. There is therefore no need to suppose any differences between paternal and maternal alleles in the target retinal tissue, and no need to invoke an influence of genomic imprinting on carcinogenesis. Consistent with this conclusion, there is no evidence in familial retinoblastoma for a difference in penetrance of the germ-line mutation, when it is transmitted through the male or female line. Such a difference would be expected if the expression or mutability of the allele differed in the target tissue.

Although the original report³ on Wilms tumour was based on analysis of only five unilateral sporadic cases, there are unpublished data showing the same findings in many others. There would be no discrepancy between the Wilms and retinoblastoma results if many or most of the Wilms tumours had originated from a germ line rather than a somatic mutation. Lack of family history may not be much help here: very few individuals with Wilms tumour, whether unilateral or bilateral, have had affected children, and so germ-line mutations at Wilms loci on chromosome 11p may be poorly transmitted. Until the Wilms gene or genes are in hand, this question cannot be resolved, and it remains an assumption that most unilateral Wilms tumours originate from sporadic mutation. Allowing this assumption for now, if the tendency for the first somatic mutation in Wilms tumour and osteosarcoma to occur on the paternal chromosome is due to genomic imprinting, presumably the Wilms locus is imprinted in embryonic kidney, and the retinoblastoma (*Rb-1*) locus in bone but not in retina.

Chromosomal regions that are probably affected by imprinting have been mapped in the mouse⁸. Comparative mapping of the homologues of the Wilms and *Rb-1* loci suggests that neither falls within regions for which there is any evidence for imprinting, but the homologous locus for Wilms tumour on mouse chromosome 2 lies between a region in which the effects of parental chromosome duplications suggest that imprinting does occur, and the homologue of the locus for the Prader-Willi and Angelman syndromes, which also show effects consistent with imprinting⁸.

A piece of evidence previously cited as consistent with imprinting at the Wilms locus is that the age at onset of tumour in cases where the gene has been inherited from the mother is greater than when inheritance is paternal. This effect is not seen in retinoblastoma. But this evidence

may no longer be relevant, because it is now known that the locus for the familial type of Wilms is not the same as the Wilms loci on 11p which have been analysed in the tumours. The difference between the results relating to somatic mutation in retinoblastoma tumours and osteosarcoma⁴ is harder to explain, because the same gene is involved in each case. Zhu *et al.*¹ raise some questions about the interpretation of the osteosarcoma data, but if the interpretation is correct, the alleles at the *Rb-1* locus in bone must differ in a way in which they do not in retinal cells.

The paternal origin of the new germ-line mutations in retinoblastoma also requires explanation. The observation is consistent with data from other inherited disorders, for example haemophilia, achondroplasia and Lesch-Nyhan syndrome. In these disorders, the incidence of new germ-line mutations rises steeply with paternal age⁹. This may be explained (at least in part⁷) by supposing that the mutations arise at DNA replication in a class of spermatogonial stem cells which are self-renewing, and which will therefore accumulate mutations with time. Retinoblastoma shows only a weak paternal age effect in comparison. Possibly, therefore, most germinal mutations at the *Rb-1* locus are of a different type, for example involving deamination at 5-methylcytosine which may be independent of mitosis, or occur in cells in which mutations do not accumulate with time. Detailed study of the mutations at the DNA level, feasible now that the *Rb-1* gene has been cloned, may provide some clues.

Perhaps the most important implication of the Wilms/osteosarcoma findings in relation to carcinogenesis is that alleles at suppressor gene loci may differ in expression or mutability within the tissue from which tumours arise. In these particular tumours, the differences may be ascribed to genomic imprinting: in other cases, perhaps differences in suppressor-gene activity could be due to polymorphisms at these loci in the general population. Such variation might contribute to inherited differences both in the risk of developing a tumour, and in the behaviour of tumours once they have developed, and might, for example, explain in part the very large variability in expression seen in many of the inherited cancer syndromes. □

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1. Zhu, K. *et al.* *Nature* **340**, 312–313 (1989).
2. Dryja, T.P. *et al.* *Nature* **339**, 556–558 (1989).
3. Schroeder, W.T. *et al.* *Am. J. hum. Genet.* **40**, 413–420 (1987).
4. Toguchida, J. *et al.* *Nature* **338**, 156–158 (1989).
5. Wilkins, R.F. *Lancet* **i**, 329–331 (1988).
6. Reik, W. & Surani, M.A. *Nature* **338**, 112–113 (1989).
7. Ponder, B.A.J. *Nature* **335**, 400–401 (1988).
8. Searle, A.G. *et al.* *Ann. hum. Genet.* **53**, 89–140 (1989).
9. Vogel, F. & Motulsky, A.G. *Human Genetics* 2nd edn 334–432 (Springer, Berlin, 1986).

SOLAR PHYSICS

How does the Sun shine?

John Bahcall

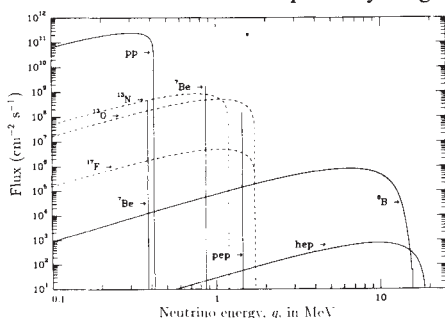
Is there something new under the Sun? Has the measurement of solar neutrinos revealed new fundamental physics or exposed poorly understood astrophysics? What can be inferred from the 10,000 accurately measured frequencies of seismological oscillations on the surface of the Sun? How accurate are the astrophysical data used in interpreting solar and stellar observations? It is difficult for a researcher working on one aspect of the Sun to be sure what is being tested using different techniques, and even more difficult to be certain what has been established firmly and what requires further investigations, which is why astronomers, astrophysicists, chemists and nuclear and particle physicists gathered recently*, with high hopes, to discuss the interior of the Sun. The result was a surprising consolidation of conventional wisdom in the face of an avalanche of new experimental and theoretical results.

The standard model of the Sun makes many quantitative predictions about the solar interior, none of which was directly testable before the introduction of the techniques of observing solar neutrinos and of solar pressure (acoustic) waves, both begun in the 1960s. Some people must have left the recent meeting thinking, as researchers have been saying for two decades because of the discrepancy between the predicted and detected flux of high-energy neutrinos from the Sun, that we are on the verge of discarding the conventional wisdom about the nature of stellar interiors. Just one more factor of two improvement in the measurements will surely reveal a fundamental flaw in our current understanding. In the meantime, the theoretical calculations improve, the data become more precise and the systematic uncertainties are better understood.

New observational and theoretical results were presented on solar neutrinos, which carry information directly from the hydrogen-burning core of the Sun. The Kamiokande II collaboration reported¹ a definite measurement of the flux of the higher-energy neutrinos which arise from boron-8. The flux is about a factor of two lower than that predicted by the standard (Bahcall-Ulrich) solar model. This provides the first experimental confirmation of the solar-neutrino problem after more than two decades of consternation based upon measurements with a single (chlorine) detector. The new result is of epochal importance because it demonstrates that the observed neutrinos do come from the Sun; the neutrinos are

detected by the recoil of scattered electrons, and these are observed to move in the Earth-Sun direction. The direction of the neutrinos is not revealed in radiochemical experiments such as the chlorine experiment of Davis.

The measurement uncertainties in the scattering rate determined by Kamiokande are, however, still too large (plus or minus 30 per cent, one standard deviation) to permit secure conclusions about either new physics or the solar model. More precise results are expected in the next few months. The first results from the Soviet-American gallium detector in the Caucasus were described. An unexpectedly large



An outstanding experimental challenge for the next decade is to measure accurately the form of the solar-neutrino spectrum. The figure shows the spectrum for each of the contributions included in the standard solar model. A precise spectrum should provide a decisive test of whether the solar-neutrino problem — the discrepancy between the predicted and measured flux — is caused by our lack of understanding of the solar interior or by new physics. The shape of each contribution to the spectrum will remain the same, whatever modification is made to the standard solar model, unless non-standard electroweak effects, such as neutrino oscillations have to be invoked. (From J.N. Bahcall *Neutrino Astrophysics*; Cambridge University Press, 1989.)

background was observed, which may be removed by further experimentation. The initial experimental measurement of the solar signal, which should go a long way towards deciding in which direction the solution of the solar neutrino puzzle lies, should be available "soon".

Pure reason scored an apparent victory at the meeting. Small discrepancies between the measured and calculated frequencies of pressure oscillations observed in optical light on the surface of the Sun led different groups, especially those at the University of California, Los Angeles² and at Los Alamos National Laboratory (reported by A. Cox), following an earlier suggestion by Christensen-Dalsgaard *et al.*³, to the conclusion that the opacity of matter at moderate stellar temperatures had previously been underestimated. The suggested correction to

the commonly used tables of atomic opacities (about 15 per cent or so at temperatures of several million degrees) is confirmed by more accurate, detailed opacity calculations (C. A. Inglesias, Livermore National Laboratory). Opacities calculated for the higher temperatures characteristic of the solar interior are now in good agreement (to within 1 per cent or less) with the values used in the standard-model calculations of the solar-neutrino fluxes.

Helioseismology yields a precise, rich characterization of aspects of the solar interior. Many thousands of oscillation frequencies have been measured with a precision approaching 0.01 per cent. A comparison of solar-model calculations with some of the earliest measurements of pressure-wave frequencies showed that the depth of the solar convective zone is somewhat deeper than previously believed.

Subsequent observations of the splitting of the frequencies of different standing waves caused by the asymmetry of the solar rotation are interpreted in terms of a relatively constant rotation rate with depth below the solar surface. No evidence has yet been obtained that there are extremely strong magnetic fields or that the solar core is rotating very rapidly, two frequently discussed non-standard solutions to the solar-neutrino problem.

Pioneering calculations for the conditions in the innermost Sun (out to a fifth of a solar radius) were presented by different theorists who have used similar helioseismological data. The independently derived numerical solutions for the physical conditions differ greatly inside the solar core, indicating that the problem of determining characteristics of the solar interior from oscillation data is not solved.

Two other problems were particularly controversial. WIMPS — weakly interacting massive particles — are hypothetical objects that could account for the missing astronomical mass and explain the solar-neutrino problem. (If they exist, WIMPs can transport energy efficiently in the solar core, lowering the central temperature and the high-energy neutrino flux as well as changing the sound speed.) John Faulkner (University of California, Santa Cruz) forcefully argued that their effects can be discerned in existing pressure-oscillation and other stellar data; several other theorists could find no such evidence in the best data.

Second, an apparent increase in the neutrino flux detected by the US chlorine detector may indicate a correlation, some argued, with the sunspot cycle, which is currently approaching its maximum. Douglas Gough (Cambridge University), who combined humour with insight in his summary talk, pointed out that it is not surprising that Ray Davis (University of Pennsylvania) finds his observations of

* *Inside the Sun*, IAU Colloquium 121, Versailles, 22–26 May 1989.

possible variations highly suggestive and that I, having contributed to the standard model which gives no hint of an explanation of such variations, should find the data statistically unconvincing.

With new detectors to measure the energy spectrum and precise time dependence of solar neutrinos, and terrestrial global networks, as well as polar observations and space experiments to determine more precisely the spectrum of pressure oscillations, solar physics is in a renaissance period. Similar techniques have produced indications of oscillations on the surfaces of other stars and many are optimistic that

definitive measurements will be forthcoming shortly. To date, the most surprising thing is that not much has been shown to be definitely new under the Sun. Surely, new data will lead soon to big surprises concerning the interiors of stars. Won't they? □

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1. Hirata, K.S. *et al.* *Phys. Rev. Lett.* **63**, 16–19 (1989).
2. Korzenic, S.G. & Ulrich, R.K. *Astrophys. J.* **339**, 1144–1155 (1989).
3. Christensen-Dalsgaard, J. *et al.* *Nature* **315**, 378–382 (1985).

NATURAL POLYMERS

Spinning ties that bind

Paul Calvert

SPIDERS make five different kinds of silk for making the web scaffolding, the sticky capture spiral, the egg sac, the attachment disks and wrapping bands for prey. Each type of silk seems to come from a specific spinning gland, although it is actually rather hard to tell which spinneret a spider is using at any one time¹. Amino-acid analyses show that there are differences among the various silks, but composition is not a good guide to polymer properties and so says little about why the silks have different functions. On page 305 of this issue², Vollrath and Edmonds discuss the origin of the elasticity of the capture spiral when compared with the stiffness of the radial frame threads.

The frame thread is a single filament of about 1 µm thick, whereas the capture thread is a pair of filaments of 0.7 µm thick with a coating of sticky (viscid) fluid. The capture thread must have many of the properties of chewing gum. It must be adhesive, it must extend to very large strains without breaking, and it must have a high tensile strength. A strong but stiff net does not spread the load well, so threads can be broken one at a time by a determined captive. As was pointed out by Gordon³, many biological materials

show a J-shaped stress-strain curve. The initial modulus is very low, but the material becomes progressively difficult to extend so that the stress rises rapidly before breaking occurs. Skin is one example of this combination of softness and strength. Vollrath and Edmonds² show a similar curve for the catching spiral in their Fig. 2a on page 306, where the 'J' has a very long tail before rising at 3 times the original length.

This long tail is achieved by the coiling of the capture thread within droplets of the coating fluid. A long, thin thread of liquid is unstable (the Rayleigh instability) and should break up into a series of drops. This happens to a thin stream of water flowing from a tap. As seen in Vollrath and Edmonds' Fig. 1 on page 306, the thread is apparently well wetted by the coating and so coils up inside the drops until it is taut in the frame. The initial large extension thus results only from uncoiling of the thread.

In the next stage of extension, the thread itself is stretched. The central argument of Vollrath and Edmonds concerns why this thread is softer than the frame thread. They suggest that the capture thread is plasticized by water from the viscid layer which surrounds it. The alternative would be to argue for a difference in structure or composition.

It is very hard to estimate the moduli of fibres because it is hard to measure their diameter. From the data presented by Vollrath and Edmonds, the modulus of the capture thread is in the range of $1\text{--}5 \times 10^7$ pascal when wet and about ten times this when dry, whereas the dry frame thread is about 10^{10} pascal. For

comparison, Nylon, which is close in composition to silk, has a modulus of 10^9 pascal as spun and 5×10^9 pascal when it has been drawn (stretched) into a textile fibre with its molecular chains oriented parallel to the axis. Like Nylon, silk does soften in a humid environment. Water breaks up the hydrogen bonding between chains and so reduces the modulus. Gosline and co-workers⁴ have shown that this effect is large in spider dragline silk (which is similar to the web frame). Exposure to water reduces the modulus from 10^{10} to 10^7 pascal. As with most textile fibres, the silk structure is a blend of crystalline and amorphous regions. The water has little effect on the crystals but converts the hard amorphous regions into a soft rubber. The water weakens, but does not eliminate, the inter-chain bonding, and the material shows viscoelasticity (slow recovery after stretching). A similar effect is seen in cold rubber. Vollrath and Edmonds go on to show that the viscoelastic responses of wet and dry frame and spiral silks are similar.

We now have a number of possibilities. First, the core of the capture thread is the same as the frame, but kept moist by the viscid coating. This could not last for a long time as the thread is very fine and must dry rapidly. Spiders spin their webs just before dawn and so they may well lose efficiency as the air dries out. In this case, the composition of the thread would be the same as for the frame. It would help to know whether the frame and spiral core came from the same spinneret but this seems to be uncertain¹.

Second, the capture thread has a different composition, which makes it more hydrophilic and so more able to retain water and be plasticized when the frame thread dries. Gosline *et al.* found that the dragline thread is stiff at 20 °C and 50 per cent humidity.

Third, the capture thread has a more irregular structure which makes it less crystalline. The greater amorphous content would make the thread more rubbery. This could be achieved with little or no change in amino acid composition. And finally, the capture thread is the same composition as the frame but is spun into a state of lower orientation.

Vollrath and Edmonds argue for the first case. It would be nice to know more, but funding in this area is not abundant. The way to resolve the problem may be to find a major application for webs — perhaps a missile defence system? □

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1. Witt, P.N., Reed, C.F. & Peakall, D.B. *A Spider's Web. Problems in Regulatory Biology* (Springer, New York, 1968).
2. Vollrath, F. & Edmonds, T. *Nature* **340**, 305–307 (1989).
3. Gordon, J.E. *Structures, Or Why Things Don't Fall Down* (Penguin, Harmondsworth, 1978).
4. Gosline, J.M., Denny, M.W. & DeMont, M.E. *Nature* **309**, 551–552 (1984).

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Beauty from strength. The silk filaments of the orb weaver's web catch the morning dew. (Courtesy of Bruce Coleman Limited.)

NEUROSCIENCE

Patch-clampers clean the brain

Philippe Ascher

THE emergence of patch-clamping¹ has undoubtedly been one of the main events in cell physiology in the past 10 years. The most spectacular aspect of this technique was the recording of single-channel currents — the fulfilment of a dream for biophysicists — but it also represents the culmination of a long effort of cell physiologists to achieve control of both extracellular and intracellular solutions. However, there were some limits to the spectacular success of the method when applied to the central nervous system. Because sealing of a patch-clamp pipette requires that the cell surface be both clean (smooth) and visible at high magnification (to allow the delicate 'landing' of the pipette tip), patch-clampers focused their efforts on neurons which could be dissociated and/or kept in tissue culture. With a few exceptions²⁻⁴ they avoided brain slices which, at the same time, were becoming established as a powerful tool for the *in vitro* study of central synapses. This barrier is now lifted by the method developed by Edwards *et al.*⁵, which allows the patch-clamping of neurons in brain slices.

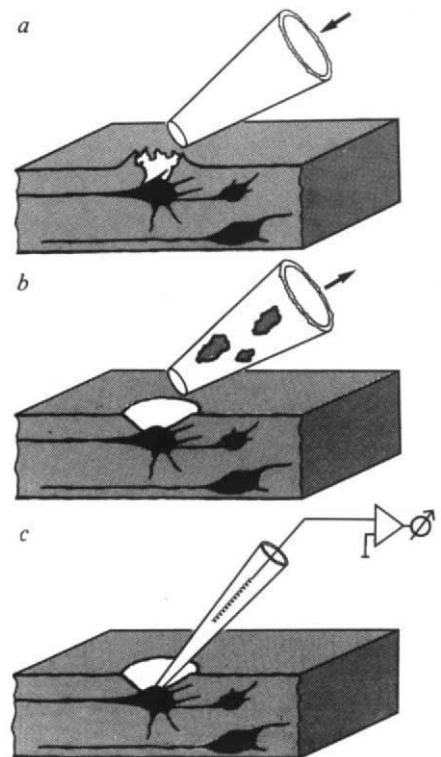
The method devised by Edwards *et al.* is surprisingly simple and comprises two steps: cutting the slice and cleaning a neuron. The slices are cut thinner (about 150 μm) than the classical slices (about 400 μm). This increases the risk of damaging neurons (particularly the delicate, branching dendritic trees), but greatly improves the quality of the image obtained with Nomarski optics. The use of thin slices has previously been advocated as a way to improve the identification of neurons⁶ and is one of the key advantages of organotypic slices⁷. Note, however, that any new method of improving visualization of the surface could relax the requirement for

thinness and allow the use of classical slices.

The second step is the cleaning, which is done without enzymes (see figure). Takahashi noted a few years ago that blowing a stream of physiological solution onto a neuron from a broken pipette was often sufficient to remove the debris covering the surface of the neuron. Edwards *et al.*⁵ find that cleaning is improved if alternated with suction, which prevents debris from being deposited. Because the cleaned surface can be restricted to a few square micrometres, the neuron under study will not float away from the slice as it can do after enzymatic treatment.

The new method can in principle be applied to slices taken from any region of the brain, and thus should allow patch-clamping of any neuron provided that it can be properly identified and is not covered by a non-neuronal cell. In addition, the method allows the study of identified synapses which can be activated by stimulation of identified tracts but also, it seems, by direct stimulation of a presynaptic neuron. This should allow analysis of quantal mechanisms, of potentiation, of depression and so on, in unrivalled conditions of control of the extra- and intracellular solutions. The new method should, furthermore, end the debate between 'patch-clampers', who advertised the fine resolution of their techniques, and 'slicers', who stressed that cultured neurons are often poorly identified, possibly de-differentiated, and that the synapses that form in culture may have little in common with those in a normal brain.

Will patch-clamping in slices provide an answer to outstanding questions in *in vitro* electrophysiology of the central nervous system? Some serious problems remain.



Patch-clamping involves the use of a glass micropipette (0.5 μm diameter) filled with current-conducting saline solution, into which a small patch of plasma membrane, which must be clean, is sucked. The patch membrane is depolarized and clamped at a set voltage and the flow of ionic current across the patch monitored by an electrode connected to an amplifier. The new technique of Edwards *et al.*⁵ involves cleaning the surface of a neuron contained in a slice by alternately blowing (a) and sucking (b) solution through a pipette placed above the target. The patch pipette can then be sealed onto the exposed neuronal surface (c). (Courtesy of Dr B. Sakmann.)

First, as patch-clamping implies replacement of intracellular media by artificial solutions, it is possible that critical intracellular components are washed away. In some cases, these factors can be added back by placing cellular extracts on the internal surface⁸. In other cases, washout can be minimized by methods such as the perforated whole-cell method^{9,10}. Washout may also be a problem in the extracellular space, because both the thinning of the slice and the cleaning of the neuronal surface may open the extracellular space in an unphysiological way, depleting important extracellular molecules or preventing their accumulation. The problem may be less severe, however, inasmuch as the method of cleaning can restrict the exposed surface.

Finally, the most difficult problem could be to control the voltage of neuronal processes such as dendrites and axons. Dendrites are probably the main site of transmission at excitatory synapses, but synapses are also known to occur on axons. Predicting what happens in distant

100 years ago



An Earthquake?

ON Friday, July 5, the inhabitants of Lyme Regis were much astonished by some noises, which took place at intervals between 11 and 11.15 p.m., and which there seems good reason to believe were caused by an earthquake. In three houses the occupiers thought that heavy pieces of furniture were being moved about, which was of course found not to be the case; and in another the inmates thought at first that something was wrong with the kitchen boiler.

The noises observed consisted of a distant rumble which grew nearer till at last the windows of the houses rattled, and in some cases distinct vibrations of the houses were felt. Some have supposed that these noises were caused by guns at sea, but this seems impossible, because (1) the rattling of the windows occurred after the distant rumble, and not simultaneously as would have been the case with guns; (2) a gentleman who has had much experience in guns and firing, has declared that the noise was not like guns; (3) after making enquiries we have been unable to discover that any firing at sea took place that night; (4) although the night was still, a heavy ground swell was observed. These phenomena have not received any notice as far as we know in the public press, and it seems a pity, if an earthquake, as we believe, really took place, that there should not be some record of it.

A. R. SHARPE.

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branches using recordings made in the cell body is difficult, and it is possible that dendritic receptors are different from the more accessible somatic receptors. Because of their thinness, most processes, even when clean and visible, may remain beyond the grasp of even the most

experienced patcher. This could be the next challenge, perhaps awaiting another technical breakthrough. □

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1. Hamill, O.P. *et al.* *Pflügers Archs ges. Physiol.* **391**, 85–100 (1981).
2. Gray, R. & Johnston, D.J. *Neurophysiol.* **54**, 134 (1985).
3. Barnes, S. & Werblin, F. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1509–1512 (1986).
4. Llano, I. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **85**, 3221–3225 (1988).
5. Edwards, F.A., Konnerth, A., Sakmann B. & Takahashi, T.

- Pflügers Archs ges. Physiol.* (in the press).
6. Llinás, R. & Sugimori, M. *J. Physiol. Lond.* **305**, 171–195 (1980).
 7. Gähwiler, B.H. *Trends Neurosci.* **11**, 484–489 (1988).
 8. Dufy, B. *et al.* *Biochem. biophys. Res. Comm.* **137**, 388–396 (1986).
 9. Lindau, M. & Fernandez, J.M. *Nature* **319**, 150 (1986).
 10. Horn, R. & Marty, A. *J. gen. Physiol.* **92**, 145–159 (1988).

PREHISTORIC ART

Hunting for farmers?

Paul G. Bahn

THE levantine rock art of eastern Spain, renowned for its depictions of game, animated human figures and hunting scenes, has traditionally been assigned to the middle Stone Age, and therefore to a culture based exclusively on hunting and gathering. New discoveries in Alicante province by Hernández *et al.*¹ provide the first clear evidence for the date of these drawings, suggesting that they began in the new Stone Age (neolithic) period, and were therefore contemporaneous with the first farmers.

Levantine art^{2,3} has long been something of a problem; it is mainly located in rock shelters and has no association with occupied sites. Breuil assigned it to the end of the last Ice Age, but recently it has been assumed to date from the mesolithic (about 8,000–5,000 BC), apparently because it often depicts hunting scenes and species such as deer and ibex drawn in a naturalistic style. It therefore served as a convenient link between the realistic animal figures of the last ice age (up to about 11,000 years ago) and the schematic and often abstract art of the neolithic and Bronze Age.

In the 1970s, Javier Fortea claimed⁴, on the basis of some engraved stones from Cueva de la Cocina dating to the epipalaeolithic (before 4,770 BC), that a 'linear-geometric' art occurred between the palaeolithic and levantine styles, and that the levantine style arose in the mid-first millennium BC.

More recently, a group of small, shallow, painted shelters has been found in Alicante province containing key superpositions of different styles. According to Hernández and his colleagues¹, a comparison with similar motifs on many objects found stratified (and hence well dated) in nearby sites produces a completely new sequence for the region's prehistoric art. It comprises a series of distinct styles, four of which are encountered in the province — palaeolithic, levantine, schematic and macroschematic,

the last being new and unique to the area.

The palaeolithic art of the last ice age is the earliest phase, found in the animal and non-figurative engravings of the Cova Fosca. The newly discovered macroschematic style is characterized by large



Macroschematic humans and meanders found in shelter V, Plá de Petracos (from ref. 1). Bar, 25cm.

human figures and by vertical and horizontal serpentiforms and meanders (see figure), all in a dense, pasty, dark-red pigment. The most striking humans have their arms raised, a pose which many assume to be one of prayer or supplication.

The only known examples of macroschematic art occur in a part of Alicante province, but the style is definitely pre-levantine because in two sites (shelter I at La Sarga and shelter IV at Barranc de Beniali) it is found under levantine figures. A study of potsherds from nearby sites (mainly old excavations at Cova de

l'Or), which bear strikingly similar depictions of human figures, assigns macroschematic art to the early neolithic and hence to the earliest communities of farmers and herders in Valencia.

Levantine art, the liveliest rock art of prehistoric Europe, occurs in shallow shelters and overhangs, and comprises mostly red (rarely black) figures: it is dominated by animals and people, either singly or in scenes. Many humans carry bows and arrows, and some goats and deer have arrows in them. Hernández and his colleagues point to analogies between this art and the goat and deer figures on potsherds at Cova de l'Or, but others find that there is little resemblance between the graceful levantine figures and the very stiff depictions seen at Cova de l'Or.

The final style, schematic art, has always been difficult to separate from levantine, especially as both tend to occur at the same sites. Figures on potsherds suggest that the former also originated in Alicante in the early neolithic, although schematic art reached its zenith in the late neolithic and Copper Age, and lasted until the Bronze Age. Figures on the wall at two sites (Abric de les Torrudanes and Abric del Barranc de la Palla) indicate that the levantine and schematic styles are at least partly contemporary.

The new findings vindicate the early work of Francisco Jordá, who first claimed⁵ that levantine art represents an agricultural and herding community which also practised hunting, rather than a pure epipalaeolithic hunting and gathering way of life. He suggested that it originated in parallel with schematic art, starting in the neolithic and developing during the Copper and Bronze Ages, but his scheme was rejected at that time.

Jordá now suggests⁶ that levantine and schematic art both arose from the macroschematic style: the former being naturalistic and narrative, the latter being more complex and elaborate, closer in style to the macroschematic, and with a far wider distribution not only in Iberia but in other parts of the Mediterranean. It is clear, nevertheless, that the startling macroschematic style dates to the early neolithic, and hence the overlying levantine art must also now be assigned to that period. The epitome of hunting art in Europe therefore began in the time of the first farmers. □

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1. Hernández, M., Ferrer, P. & Catalá, E. *Arte Rupestre en Alicante* (Banco de Alicante, 1988).
2. Dams, L. *Les Peintures Rupestres du Levant Espagnol* (Picard, Paris, 1984).
3. Beltrán, A. *Rock Art of the Spanish Levant* (Cambridge University Press, 1982).
4. Fortea, J. *Zephyrus* **25**, 225 (1974).
5. Jordá, F. *Zephyrus* **25**, 209 (1974).
6. Jordá, F. in *Arqueología del País Valenciano. Panorama y Perspectivas* 121 (1985).

Depletion on volcanic aerosols

R. L. Jones

VOLCANO eruptions can so disturb the stratosphere that they may lead to significant destruction of ozone, according to new work by Hofmann and Solomon¹. The effect was apparent after the massive eruption at El Chichon in 1982 which injected large quantities of sulphur dioxide into the stratosphere. Here, the gas condensed with water to form aerosol particles, which are the crucial catalytic sites for ozone destruction.

That chemical reactions on the surfaces of stratospheric particles can influence stratospheric composition is now widely recognized. Laboratory studies²⁻⁴ suggest, and composition measurements⁵⁻⁷ demonstrate that large perturbations to the partitioning of both the chlorine and nitrogen families of compounds can occur during the polar winter, when temperatures fall sufficiently for the formation of nitric acid-trihydrate and/or water-ice clouds⁸. In air which has been exposed to these clouds, the mixing ratios of many reactive chlorine gases, particularly chlorine monoxide and the symmetric chlorine dioxide, can be 10–100 times larger than would be expected without such exposure.

A secondary, but important additional effect of these surface reactions and particle formation is the sequestration of reactive nitrogen oxides into the reservoir nitric acid, which may then be partially removed from the low stratosphere through condensation and subsequent sedimentation. The conversion or removal of reactive nitrogen compounds slows the photochemical recovery of the reactive chlorine back to reservoir forms⁹. In the presence of sunlight, significant depletion of ozone may then occur^{9,10}.

Over Antarctica, these events have led to the development of the ozone 'hole'. In the Arctic, large perturbations to the chlorine chemistry have been noted by Solomon *et al.*¹¹ and the recent Airborne Arctic Stratospheric Expedition, the beginnings of an ozone depletion being tentatively detected¹². The extent of ozone depletion depends on the degree to which sunlight is present while the photochemistry is perturbed. In the Antarctic, the vortex remains largely intact, and cold temperatures persist, until well into spring, allowing a substantial depletion of ozone. In the less stable, warmer Arctic winter vortex, conditions for ozone depletion are less favourable.

Because of the dual requirement for cold temperatures (less than about 195 K) and sunlight, ozone depletion arising from these processes is broadly restricted to high latitudes in winter and early spring. Although cold temperatures do exist in equatorial latitudes, no equivalent ozone

depletion is thought likely to occur, because insufficient chlorine has been released from reservoir forms (mainly chlorofluorocarbons) in this air of more recent tropospheric origin to allow reactive chlorine concentrations to build up to damaging levels. Thus, at first sight it seems that severe ozone depletion involving heterogeneous reactions on stratospheric clouds is restricted to high latitudes and to spring months.

In their new paper¹, however, David Hofmann and Susan Solomon argue

Dynamical effects spread the ozone hole

MID-LATITUDE ozone levels could be threatened not only by the heterogeneous chemical effects described by R. L. Jones, but also by dynamical redistribution towards the tropics of depleted air from the polar ozone hole. It has not been clear whether the ozone hole, generated each spring in the antarctic stratosphere since 1979, does spread at the onset of summer, but Atkinson *et al.* report on page 290 of this issue¹ that the ozone concentration over Australia and New Zealand was unusually low at the end of 1987. Whether this is linked to polar depletion is of special interest to those modelling the global threat to ozone.

Ozone concentrations in the Antarctic reached a record low in the spring of 1987. Furthermore, the polar vortex — the intense westward circulation extending out to 60° S that confines the ozone hole — did not break up until early December, about a month later than usual. The record-low ozone values over populated regions in New Zealand and Australia were also observed in December. The data examined by Atkinson *et al.*¹ come from Perth, Melbourne, Brisbane and Hobart in Australia and Lauder in New Zealand, covering an area from 43° to 30° S.

From an analysis of the meteorological conditions prevalent at the time, the researchers conclude that parcels of air from the edge of the decaying vortex carried cold, ozone-poor polar air over Australia. Such a dilution effect has already been suggested by modellers (refs 2 and 3) and clearly there are potentially serious implications, although the sensitivity of this effect to changes in atmospheric dynamics has yet to be assessed.

Although the chemistry of Antarctic ozone depletion is now probably understood, dynamical activity is also important. This is illustrated by the striking differences in the characteristics and scale of the 1987 and 1988 ozone holes (see the News and Views article by Mark Schoeberl¹). It seems that the interannual fluctuations in

persuasively that following the El Chichon eruption in 1982, the ozone concentration between 10° and 50° N was significantly depleted — by approximately 15 per cent locally (their Fig. 10). This was, they argued the result of heterogeneous reactions on sulphuric acid particles injected into the stratosphere by the Central American volcano.

The main, worrying difference between their hypothesized situation and the mechanism outlined above is that the requirement for temperatures to be sufficiently low for stratospheric clouds to form no longer exists. In mid-latitudes, significant quantities of chlorine have been made available from long-lived reservoirs. The region of high concentrations of reactive chlorine,

total ozone concentrations in the Antarctic are linked to the tropical quasi-biennial oscillation⁷ with relatively low (high) values occurring during the westerly (easterly) phase of this large-scale circulation. The marked drop in ozone concentrations over Australia is therefore likely to recur when ozone levels are low in the Antarctic.

The springtime polar vortex is isolated from ozone-rich air from mid-latitudes and forms a chemical containment vessel in which the cold temperatures allow the formation of polar stratospheric clouds. These provide surfaces for heterogeneous chemical reactions, releasing active chlorine and removing odd nitrogen, thus preconditioning the vortex for ozone depletion to take place when sunlight returns in the early spring.

There is some uncertainty, however, over how isolated the vortex must be for ozone depletion to take place. Some believe that the ozone-poor air within the vortex is tightly contained; others believe that air from lower latitudes descends through this region and is transported northwards at lower altitudes, so that the lower levels of the vortex effectively leak.

With more precise calculations, it might be possible to use early-spring observations from Australasia to distinguish the two models of the Antarctic ozone hole. According to both models, ozone-rich air from the tropics is deflected down onto Australasia by the vortex. But if the vortex is a leaky vessel, a small trickle of ozone-poor air will mix with this flow throughout the southern spring, whereas a sealed vortex would retain its contents until break up.

Philippa Lloyd

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1. Atkinson, R.J., Matthews, W.A., Newman, P.A. & Plumb, R.A. *Nature* **340**, 290–294 (1989).
2. Sze, N.D. *et al.* *J. geophys. Res.* (in the press).
3. Prather, M.J. & Garcia, M.M. *NASA Conf. Pub.* 10014 (1988).
4. Schoeberl, M.R. *Nature* **336**, 420–421 (1988).
5. Garcia, R.R. & Solomon, S. *Geophys. Res. Lett.* **14**, 848–851 (1987).

where ozone depletion is threatened is thus determined by the spatial extent of the volcanic cloud and can be far more extensive. For example, the volume of atmosphere between the equator and 50° N (the approximate bounds of the El Chichon cloud) is more than an order of magnitude greater than that within the polar vortex, say poleward of 70° N. If reactions on volcanic aerosol perturb the partitioning of the nitrogen and chlorine species over such a volume, the effect on the global quantities of ozone could be considerable.

The stratospheric sulphate aerosol layer was discovered¹² in the early 1960s. In 'normal' conditions, that is in the absence of perturbation by volcanic effects, sulphur compounds reside in the stratosphere, largely in the form of sulphur dioxide and carbonyl sulphide, with mixing ratios in the range 0.1–1.0 parts per 10⁹ by volume. Both are converted photochemically to sulphuric acid vapour. Under suitable conditions, aqueous sulphuric acid droplets form on existing condensation nuclei. Once formed, the particles can grow by condensation of sulphuric acid and water vapour, and by the coagulation of particles¹³.

Larger particles can be removed by sedimentation, and at upper altitudes (above approximately 30 km) particles evaporate. The result is the formation of a layer of particles in the low stratosphere with a distinct maximum 5–7 km above the tropopause. The normal background concentration of aerosol particles is of the order of 10 per milligram of air, and the available surface area of the order of 1 $\mu\text{m}^2 \text{ cm}^{-3}$. But in the aftermath of volcanic eruptions, both may be increased by several orders of magnitude.

Following El Chichon, some $3\text{--}8 \times 10^{12}$ g of sulphur dioxide were injected into the stratosphere^{14,15}. On the basis of their measurements, Hofmann and Solomon point out that this increased the surface area available for chemical reactions from $0.75 \mu\text{m}^2 \text{ cm}^{-3}$ to $20\text{--}50 \mu\text{m}^2 \text{ cm}^{-3}$. The latter, they point out, is comparable to that observed in polar regions, which is associated with clouds of nitric acid trihydrate. They conclude using measured (or in one case an assumed) heterogeneous reaction rates on sulphuric acid surfaces, that ozone concentrations were reduced by up to 15 per cent over a substantial portion of the Northern Hemisphere. If this decline is representative of that at other altitudes where cloud particles exist (up to 28 km; ref. 16), the net atmospheric ozone reduction would be at least comparable to that observed during the formation of the Antarctic ozone hole.

As the authors state, their results depend critically on their choice of accommodation coefficients, factors which control the rate of heterogeneous conversion of chlorine and nitrogen

compounds on particle surfaces. These are clearly uncertain. By perhaps their choice of the $\text{N}_2\text{O}_5 + \text{HCl}$ reaction rate on sulphuric acid surfaces, they have tended to maximize their ozone loss. It would be instructive to examine whether the ClO mixing ratios implied by their choice can be confirmed by direct measurement.

Although the authors suggest that the consequences of massive volcanic injection can be very significant, the impact of background aerosol levels are apparently less marked¹⁷. However, it is intriguing that, when the effects of El Chichon aerosol are included, Hofmann and Solomon's modelled latitude gradient of nitric acid reproduces the gradient observed by the LIMS instrument (their Fig. 10) in January 1979, a period of much lower stratospheric aerosol concentration. Without heterogeneous effects, the 1979 observations and model calculations differ by a factor of two.

At high latitudes, heterogeneous production of nitric acid on nitric acid trihydrate particles is likely to have occurred during the cold late December and early January. However, the agreement at low latitudes implies at face value that the heterogeneous effects on the partitioning of reactive nitrogen compounds, even on background aerosol, can be large. The effect of background aerosol concentrations on the partitioning of the chlorine species may thus be large also. This would enhance the ozone loss reported by earlier studies¹⁷ which did not include the reaction of HCl with N_2O_5 .

As Hofmann and Solomon point out, rather than being interpreted as definitive, their results should be regarded as illustrative of possible effects. With increasing atmospheric chlorine concentrations, these possible effects should give serious cause for concern, particularly given uncertainties in heterogeneous reaction rates. □

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- Hofmann, D.J. & Solomon, S. *J. geophys. Res.* **94**, 5029–5041 (1989).
- Molina, M.J., Tso, T.L., Molina, L.T. & Wang, F.C. *Science* **238**, 1253–1259 (1987).
- Tolbert, M.A., Rossi, M.J. & Golden, D.M. *Geophys. Res. Lett.* **15**, 851–854 (1988).
- Leu, M.T. *Geophys. Res. Lett.* **15**, 17–20 (1988).
- Solomon, S., Mount, G.H., Sanders, R.W. & Schmeltkeopf, A.L. *J. geophys. Res.* **92**, 8329 (1987).
- Fahey, D.W. *et al. J. geophys. Res.* (in the press).
- Brune, W.H., Anderson, J.G. & Chan, K.R. *J. geophys. Res.* (in the press).
- Toon, O.B., Hammill, P., Turco, R.P. & Pinto, J. *Geophys. Res. Lett.* **13**, 1284 (1986).
- Jones, R.L. *et al. J. geophys. Res.* (in the press).
- Anderson, J.G. *et al. J. geophys. Res.* (in the press).
- Solomon, S. *et al. Science* **242**, 550–555 (1988).
- Junge, C.E., Chagnon, C.W. & Manson, J.E. *J. Meteorol.* **18**, 81–108 (1961).
- Turco, R.P., Whitten, R.C. & Toon, O.B. *Rev. Geophys. Space Phys.* **20**, 233 (1982).
- Krueger, A.J. *Science* **220**, 1377–1379 (1983).
- Thomas, G.E., Jakosky, R.A., West, R.A. & Sanders, R.W. *Geophys. Res. Lett.* **10**, 997 (1983).
- Reiter, R., Jager, H., Carnuth, W. & Funk, W. *Geophys. Res. Lett.* **10**, 1001 (1983).
- Rodriguez, J.M., Ko, M.K.W. & Sze, N.D. *Geophys. Res. Lett.* **15**, 257–260 (1987).

The fabric of life

LAST week Daedalus presented 'Livewear', a novel form of clothing in which an inert base-fabric supports a fibrous mat of self-renewing moss or similar vegetation. Moistened by the constant slight sweating of human skin, and absorbing dirt and grime as a fertilizer, it cleans and repairs itself, and even grows, in normal use.

Daedalus is now seeking to extend this principle to other fabrics. Living wash flannels, napkins and tablecloths would doubtless pick up enough water and nutrients in normal careless domestic use; but biological carpets, curtains and so on would wilt and die from lack of moisture. But Daedalus recalls the Lomas vegetation of Peru, which draws moisture out of the air and can grow in regions where rain never falls. Some plants can work this trick even in quite unsaturated air.

So DREADCO's biologists are playing the usual breeding and mutation games: crossing promising varieties with each other, exposing them to strong ionizing radiation, and so on. They hope to develop a plant variety or stable ecosystem which not only forms a coherent fibrous growth on a base fabric, but extracts enough water from the air to survive on its own.

The new 'Phytomat' will have many uses. As a domestic fabric in rugs and curtains and bedding, and so on, it will give the house a delightful natural look. It will never need renewing or cleaning; and it will keep the rest of the house clean as well, by absorbing and metabolizing household dust and dirt (which is mainly organic). One problem may be its tendency to spread. Even the smallest Phytomat rug in a room might in time colonize the whole room, covering floor, walls, ceiling and furniture with a soft, verdant, insulating living layer. Even the occupants themselves, or at least their clothing, could be at risk. The DREADCO team may have to restrict the seeding cycle of their new product, or devise a special herbicidal coating to keep it in bounds.

On the other hand, a self-spreading mossy vegetable mat could be a wonderful land reclamation system. A big roll of a suitable Phytomat fabric could simply be spread over some dusty patch of wasteland and left. Absorbing moisture from the air and nutrients from the soil, it would soon establish itself as a primary ecosystem, ultimately to be built on and replaced by the richer and more varied ecosystem natural to the area. Dustbowls could be reclaimed and deserts colonized. Daedalus even has plans for a special Phytomat garden for lazy householders, 'printed' in tasteful patterns with the seeds of grasses, shrubs and flowers. Just roll it out over your neglected yard, and in due course a perfect garden will arise, the envy of the neighbours.

David Jones

Rock avalanche run-up record

SIR—During movement along its path, rock-avalanche debris can exhibit spectacular mobility in surmounting substantial topographic obstacles¹, in running up opposing slopes², and in the superelevation of its surface in valley bends in its path². In investigating the run-up at the Avalanche Lake rock avalanche located at 62° 25' N, 127° 15' W in the uninhabited Mackenzie Mountains of the Cordillera of western Canada, I have documented a run-up that is both highly anomalous and the highest ever recorded.

The avalanche was first reported by Gabrielse *et al.*³. It is one of several highly mobile rock avalanches in the Mackenzie Mountains described by Eisbacher⁴ and is the most recent of multiple rock avalanches near Avalanche Lake. It involved the detachment of an estimated $5-6 \times 10^8$ m³ of Palaeozoic limestone along a remarkably planar bedding surface dipping at about 31° S towards the valley (see figure). The disintegrating rock mass descended into the valley where most of it was deposited, forming a dam that impounded Avalanche

Lake. Some of the debris, however, travelled over the valley floor and up the precipitous south side of the valley (the wall) to elevation 1,640 m above sea level. It then spread out on a feature called the shelf, running up a further 30–60 m at its southern margin. The deposits are called the shelf lobe.

It can be estimated from part *a* of the figure that the height of the descent slope, h_1 — the elevation difference between the top of the headscarp and the bottom of the pre-landslide valley — is 1,220 m and that the maximum height of the run up, h_2 , is 640 m. Using the simple velocity head formula⁵, $v^2 = 2gh$ (v = velocity, g = acceleration due to gravity and h = height of run-up), which does not take into account frictional losses during movement, a velocity of 112 m s⁻¹ is calculated for the debris at the base of the wall. An expression derived by Francis and Baker⁶, which does take into account frictional losses, yields a velocity of 213 m s⁻¹.

The magnitude of the run-up at Avalanche Lake is not only the highest

recorded but is also highly anomalous in relation to h_1 . A plot of h_1 against h_2 for 24 rock avalanches from around the world (unpublished data) shows that a line equivalent to $h_2 = 0.3 h_1$ clearly defines an upper limit for the data. But for the Avalanche Lake data $h_2 = 0.52 h_1$.

During field work at Avalanche Lake in 1987, numerous wood fragments were found scattered throughout the debris of the shelf lobe. Typically, the fragments consisted of broken logs and splinters and were wedged beneath or between boulders in the debris. Splinters were also found at the strand line of the secondary run-up at the southern margin of the shelf. The shelf is above the tree line and no tree fragments were found beyond the limits of the shelf lobe debris. The nature and the distribution of the wood fragments clearly suggests that they were incorporated into the debris as the avalanche smashed through trees during its travel over the valley floor of the main Avalanche Lake valley, which is below the current tree-line and which sustains scattered tree-growth beyond the debris (part *b* of figure).

Radiocarbon dating (by the Radiocarbon Laboratory of the Geological Survey of Canada) of four fragments of wood, one of which was collected by P.K. Kaiser and J.V. Simmons, yields a youngest date of 320 ± 50 yr before present. Using the calibration curves of Stuiver and Pearson⁷, this date indicates that the avalanche occurred no earlier than 1450 AD.

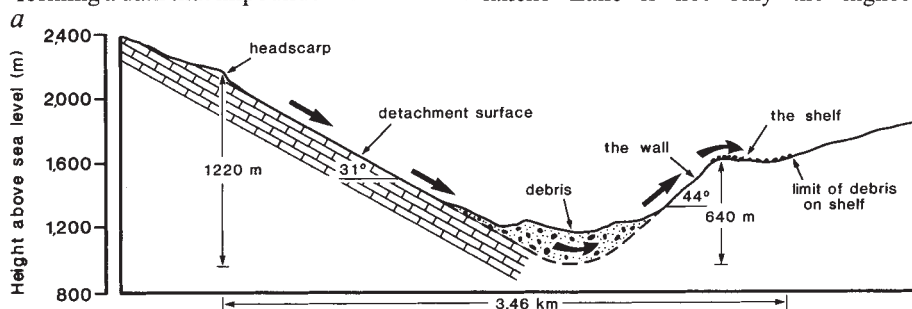
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1. Harrison, J.V. & Falcon, N.L. *J. Geol.* **46**, 296–309 (1938).
2. Evans, S.G. *et al.* *Can. geotech. J.* (in the press).
3. Gabrielse, H. *et al.* *Geol. Surv. Can. Memoir* **366**, 153 (1973).
4. Eisbacher, G.H. *Can. Geotech. J.* **16**, 309–334 (1979).
5. Chow, V.T. *Open Channel Hydraulics* (McGraw-Hill, New York, 1959).
6. Francis, P.W. & Baker, M.C.W. *Nature* **270**, 164–165 (1977).
7. Stuiver, M. & Pearson, G.W. *Radiocarbon* **28**, 805–838 (1986).

Calcium oscillations

SIR—Wakui *et al.*¹ report that InsP₃, a non-metabolized analogue of 1,4,5-inositol trisphosphate (InsP₃) can induce pulsatile intracellular release, deduced from a measured pulsatile increase in Ca²⁺-dependent chloride conductance. The InsP₃ was introduced into mouse pancreatic acinar cells via a 'whole-cell' patch pipette and presumably achieved a steady intracellular level over the many minutes of the experiments. The authors conclude that intracellular Ca²⁺ oscillations (at least in mouse pancreatic cells) are not generated by "spiking levels of InsP₃" and state in their title that "pulsatile intracellular release does not depend on fluctuations in



a, Profile of the path of the Avalanche Lake rock avalanche based on 1:50,000 topographic map 95L/6. The pre-landslide topography of Avalanche Lake valley was estimated by projecting the thalweg of the unnamed river that occupies the valley beneath the debris. *b*, Oblique aerial view (to the west) downstream of Avalanche Lake (A), showing debris in the valley (D), the shelf (S), the wall (W) and a fallback ridge (F) at the wall's base. Debris can be seen on the shelf and secondary run-up, the upper limit of which is delineated by the dashed line, at the shelf's southern margin.

inositol trisphosphate concentration".

The data in this report are clear-cut, and are based on an elegant and novel approach using a purpose-built chemical variant of InsP_3 . But the authors' conclusions are not the only, or necessarily the most likely, interpretation of their results.

One alternative is that InsP_3 acts as a stimulus for a small release of intracellular Ca^{2+} to raise intracellular calcium concentration to a level below the threshold for increased chloride conductance, but enough to activate, or increase the basal activity of, phospholipase C and hence generate endogenous InsP_3 . The stage would then be set for intracellular Ca^{2+} spiking based on feedback between InsP_3 -induced Ca^{2+} discharge and Ca^{2+} -enhanced phospholipase C activity as modelled by Meyer and Stryer². We know from Fig. 1 of Wakui *et al.*¹ that their cells are capable of generating spikes in response to acetylcholine, a ligand acting via the inositol lipid signal pathway.

The analysis of Wakui *et al.* is based on the idea that a stimulus cannot be part of the feedback generating a train of spikes if a steady application of that stimulus evokes spikes. But this is like arguing that generation of a train of action potentials in a motor neuron cannot be based on inward currents and membrane depolarization, because such trains can be initiated experimentally by application of depolarizing current via a microelectrode. Wakui *et al.* seem to have neglected the possibility that InsP_3 could have activated the cells to generate their own InsP_3 (and possibly also InsP_4). We need measurements of inositol phosphates, in single cells on the appropriate time scale, before deciding whether they do or do not fluctuate during intracellular Ca^{2+} oscillations³.

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PETERSEN *ET AL.* REPLY—Rink's attempt to resurrect the theory that pulsatile inositol (1,4,5) trisphosphate (InsP_3) production is responsible for intracellular Ca^{2+} spikes involves a complicated explanation and an unlikely assumption. Our data¹ show that inositol (1,4,5) trisphosphorothioate (InsPS_3) evokes the same effect as InsP_3 . Because it has been clearly shown that InsPS_3 , although about 3–5-fold less potent than InsP_3 , is nevertheless a full agonist for the release of Ca^{2+} from intracellular stores^{4,5}, why then assume that in our experiments InsPS_3 evokes a "small release of intracellular Ca^{2+} " and that its demonstrated effect is due to the secondary production of InsP_3 ?

The mechanism postulated by Rink is Ca^{2+} -activation of phospholipase C. But in pancreatic acinar cells this process seems

to be of little significance, as secretagogue-evoked InsP_3 production is independent of changes in intracellular Ca^{2+} concentration⁶ and stimulation of intact cells with the Ca^{2+} ionophore A23187 results in very little secretion unless coupled with either a muscarinic agent or an activator of protein kinase C (ref. 7). These results indicate that a Ca^{2+} signal alone cannot elicit significant phosphatidyl inositol (4,5) biphosphate hydrolysis and therefore diacylglycerol production.

Our data¹ do seem to show that InsPS_3 is perhaps about 2–3-fold more potent relative to InsP_3 than had been expected on the basis of experiments in permeabilized cells^{4,5}, but this can be explained very simply, as InsP_3 would be subject to breakdown in our cells, whereas this would not be the case for InsPS_3 .

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1. Wakui, M., Potter, B.V.L. & Petersen, O.H. *Nature* **339**, 317–320 (1989).
2. Meyer, T. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5051–5055 (1988).
3. Rink, T.J. & Jacob, R. *Trends Neurosci.* **12**, 43–46 (1989).
4. Taylor, C.W., Berridge, M.J., Cook, A.M. & Potter, B.V.L. *Biochem. J.* **259**, 645–650 (1989).
5. Strupish, J., Cooke, A. M., Potter, B. V. L., Gigg, R. & Nahorski, S. R. *Biochem. J.* **253**, 901–905 (1988).
6. Streb, H., Heslop, J.P., Irvine, R.F., Schulz, I. & Berridge, M.J. *J. biol. Chem.* **260**, 7309–7315 (1985).
7. De Pont, J.J.H.M. & Fleuren-Jakobs, A.M.M. *FEBS Lett.* **170**, 64–68 (1984).

Amylin hormone

SIR—Betsholtz *et al.*¹ have recently commented on our use of the name amylin^{2,3} for the newly discovered 37-amino-acid peptide hormone which is found in β -cells of the pancreatic islets of Langerhans and probably secreted along with insulin. This peptide has also been termed DAP⁴ (diabetes-associated peptide). The primary amino-acid sequence of amylin is known, its carboxy terminus is amidated, and it is a potent down-regulator of both basal and insulin-stimulated glucose uptake and glycogen synthesis in mammalian skeletal muscle *in vitro*^{2,3}.

Betsholtz and colleagues have isolated from insulinoma-associated amyloid a

peptide called IAP⁵ (insulinoma amyloid peptide) or IAPP⁶ (islet or insulinoma amyloid polypeptide) which is similar to amylin in primary sequence. The equivalence of IAP/IAPP and amylin remains to be proved, however, as the complete sequence and structure of IAP/IAPP have not been determined, nor has any functional activity been reported for either. It should be noted that a change at a single amino-acid locus may result in a variant, amyloidogenic protein which is able to form amyloid deposits⁷.

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Radiation limits

SIR—Robin Russell Jones¹ argues that public radiation dose limits should be reduced to 0.2 millisievert (mSv) per year. This refers to the additional radiation doses to the public from industrial sources. The limit is currently 1 mSv a year if exposure is prolonged, and the UK National Radiological Protection Board has recommended reduction to 0.5 mSv. In practice, the average additional dose due to such sources is 0.001 mSv a year, and the highest dose to the public recorded in 1987 was 0.33 mSv, with very few members receiving more than 0.1 mSv (ref. 2).

These doses are very low compared with the total exposure to radiation³. The average annual UK dose is 2.5 mSv, and 7.8 mSv in Cornwall. The highest doses, which can exceed 50 mSv a year, are due to indoor radon, and the highest artificial doses are due to medical exposures, which average 0.3 mSv a year. Both of these could be reduced by remedial measures^{4,5}.

The low doses from industrial sources have been achieved by heavy expenditure. It is time to concentrate our radiological protection measures on that part of the population at highest risk rather than to attempt to reduce even further the small additional doses to groups which are already at very low risk. Exposures to ionizing radiation should be considered in total.

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1. Betsholtz, C., Johnson, K.H. & Westermark, P. *Nature* **338**, 211 (1989).
2. Cooper, G.J.S. *et al. Proc. natn. Acad. Sci. USA* **85**, 7763–7766 (1988).
3. Leighton, B. & Cooper, G.J.S. *Nature* **335**, 632–635 (1988).
4. Cooper, G.J.S. *et al. Proc. natn. Acad. Sci. USA* **84**, 8628–8632 (1987).
5. Westermark, P., Wernstedt, C., Wilander, E. & Sletton, K. *Biochem. biophys. Res. Comm.* **140**, 827–831 (1986).
6. Westermark, P. *et al. Proc. natn. Acad. Sci. USA* **84**, 3881–3885 (1987).
7. Yoshioka, K. *et al. Molec. biol. Med.* **3**, 319 (1986).

1. Russell Jones, R. *Nature* **339**, 20 (1989).
2. Hunt, G. J. *Radioactivity in Surface and Coastal Waters of the British Isles, 1987* (MAFF, Lowestoft, 1988).
3. Clarke, R.H. & Southwood, T.R.E. *Nature* **338**, 197 (1989).
4. *Exposure to Radon Daughters in Dwellings* NRPB-GS6 (National Radiological Protection Board, Chilton, 1987).
5. Russell, J.G.B. & Webb, G.A.M. *Brit. J. Radiol.* **60**, 681 (1987).

Back to Green roots

Richard Mabey

Ecology in the 20th Century: A History. By Anna Bramwell. Yale University Press: 1989. Pp. 292. Hbk £27.50, \$40; pbk £9.95, \$14.95.

ANNA Bramwell and Yale University Press must be blessing the serendipity — or inspired foresight — that made the publication of this first “intellectual and political history of the ecology movement” coincide with the extraordinary successes of the Greens in the European elections. But in another way it is less timely, and appears to have been sent to the press before the greenhouse effect, deforestation and oceanic pollution became such crucial, converging political issues. It is a moot point whether these matters would have been thought relevant to a book whose tone is largely that of a seminar conducted in a windowless room. But they might have made the author less shrilly dismissive of Green thinking in her conclusion. Here she repeats an increasingly familiar critique of the Greens’ supposedly austere and backward-looking philosophy, but adds a personal note of quite exceptional bitterness — which may be a clue to the baffling selectivity of a book that is otherwise mostly a detached, albeit tortuous, academic study.

Dr Bramwell’s thesis is that Green philosophy — or “ecologism” as she insists on calling it — is a quite new political axis. It cannot be reduced to some version of traditional left or right ideologies, and is distinct from scientific ecology (some scientists who feel their discipline has been appropriated would agree). Its real roots lie in the nineteenth century, in the meeting between holistic biology and “resource-scarcity economics”. One question that persistently nags her is whether the Nazis were early ecologists (her previous book was *Blood and Soil: Walther Darré and Hitler’s Green Party*) and it is no surprise that she identifies the ‘founding father’ of ecologism as the German biologist Ernst Haeckel (1834–1919). Haeckel is often said to have invented the term ‘ecology’ (though Thoreau used it ten years before him) and — the author brushes past the circularity of this argument — he fulfils most of her criteria for a true believer. He had a “total

world-view which does not allow for piece-meal reform... fears the dissipation of energy and resources, and is not anthropocentric”. And by his foundation of the Monist League and influence on the Soil Association, he is at the grass roots of the intellectual network whose development into the modern Green movement Bramwell attempts to trace.

This eliding of a ‘current of thought’ and an actual, historical movement is



Elected Greens — Petra Kelly (left), then chairwoman, and Lucas Beckmann, then party secretary, address a news conference in Bonn, West Germany, following the success of the Greens in the 1982 Hesse state elections.

characteristic of many unjustified assumptions that underlie Dr Bramwell’s argument. Persistently she equates ecologism with “ruralism” and “the smallholding idea”. She believes that the notion of a primal scapegoat or “wrong turning” — be it industrialism, the shift from pastoral to agriculture, or just plain *men* — is essential to Green ideology. And most centrally she insists that the strength of ecological ideas “is not directly linked with actual problems” — an assumption comparable to saying that the growth of socialism had nothing to do with the development of factories.

These are the criteria that lie behind her idiosyncratic voyage through the backwaters of Western Utopian thinking. She corrals Konrad Lorenz, and his belief that understanding man’s animal nature would

help solve his political and social problems; revives the vitalist ideas of Schrödinger, who defined organic life as that which resisted entropy; and uncovers an early Gaian in A.J. Herbertson, who in 1905 described the Earth as a “macro-organism... the soil itself the flesh, the vegetation its epidermal covering”.

Herbertson’s metaphor was benign and neutral. But in 1930s Germany, discussion of which occupies a sizeable portion of the book, fertile soil and unpolluted water became symbols of the virtue of the uncorrupted, hard-working, racially-pure human being in close touch with his fatherland. In Britain at least, the skein of Green threads teased out by Dr Bramwell has less of this sinister piety. But it often reflects its paternalism. The early decades of this century saw a tumult of libertarian

and ‘organic’ theorizing, and even some real-life experiments. Kropotkin’s decentralist ideas were based on the similarly diverse and mutually aiding ‘laws of the environment’. The elegiac socialist Robert Blatchford agreed, but thought that only by a planned intensification of agriculture could Britain be self-supporting. The idea of benevolent planning stretched from George Stapledon’s grassland improvement campaign to the radical geographers’ ideas for a more equitable way of resettling the planet. And in the outer shades of these green thoughts Dr Bramwell finds the Boy Scout Movement, human eugenics, Tolkien, biodynamic farmers, feminist moon worshippers and just about the whole New Age movements.

There is much that is perceptive here, especially the tracing of a long history of ambivalence towards science (viewed both as culprit and cure) and towards the paradox of planning for an ‘organic’ society. Yet it seems increasingly remote from what we understand as the body of Green ideas, and more the results of a spell of beachcombing along the wilder shores of ‘alternative’ and Utopian thought. Dr Bramwell is trying to uncover a taxonomy, but is really chancing upon examples of convergent evolution.

Modern Green thinking is more specific, more vernacular and vastly more pragmatic than she acknowledges. To the extent that its adherents recognize an intellectual ancestry, in Britain it would be more likely to include David Bellamy, the Campaign for Real Ale and Wordsworth than obscure Teutonic vitalists. But it is

the insistence that the 'strength of ecological ideas' has nothing to do with real-life problems that is the central fallacy of her book. No body of political ideas has been *more* based on what is happening in the world. At the individual level of, say, a pesticide allergy or lost hedgerow to the global catastrophe of Chernobyl, the springboards for Green political consciousness are not only 'actual problems' but actual experiences, which lead to a real, often physical understanding of the interconnectedness of life. If one wished to conceptualize, this 'greening' could be seen as a sophisticated form of biological feedback.

Perhaps the most significant of these experiences was the image of planet Earth from space, which the biologist Lewis Thomas described as being like the vision of a single cell. Dr Bramwell asserts that "American ecologists... have ignored their ancestors", and then ignores them herself. It is a strange claim, given the depth and realism of the New World tradition, but perhaps it is their very rootedness in real problems that causes Dr Bramwell to omit them — the English-born Charles Waterton's passionate defence of the South American rain forests in the 1820s; John Muir's writings on the value of wilderness; Aldo Leopold's 'land ethic', which is the single most influential idea in the whole ecological canon; Barry Lopez on the political ecology of the Arctic.

It is these sensible biologists, as well as the more overtly political thinkers who have found a congenial home with the Greens, that stand accused by Dr Bramwell's astonishing and at times barely coherent outburst in her closing pages: "[the movement] still carries the burden of its heritage, the legacy of the crucifixion, symbol of death, suffering and self-surrender... their hope of regeneration presupposes a return to primitivism... the burning before the replanting". This is the language of the witchhunt, and it is strange to find it loose on a movement most of whose followers view its philosophy more prosaically, as an extension of the principle of the bottle-bank.

It is sad that the book ends in this way, because Dr Bramwell does begin to touch on many of the shortcomings of the *organized* Green movement. Like all minority groups that have not experienced political power, it does have a tendency towards authoritarianism, to wagging a theoretical finger at the messy complexity of human affairs. There is a residual puritanism, too — traceable back to the English Revolution, if it does have historical roots — in a distaste for private property and a belief in the redemptive as well as economic value of manual work. (A recent editorial in *Environment Now* described labour as "the ultimate renewable resource.") And although Dr Bram-

well's jibe of primitivism is plain silly, a similar confusion between aesthetic and strictly ecological considerations is the Greens' main weakness when it comes to the contentious issue of economic growth. A distaste for rampant consumerism becomes inflated to a belief that the Earth's resources are static — which of course they aren't. Although its living space and basic elements may be fixed, its energy levels are not, and the ecosystem's great strength has been to find an infinite variety of ways of rearranging these. The game of economics can be played *within* these constraints; and no one would say, for instance, that the economic growth represented by massive reafforestation would be ecologically undesirable.

Dr Bramwell's concluding argument is so strident that one begins to ask questions about her own intellectual pedigree. Her writing is something of a paradox: seemingly meticulously researched, yet riddled with small errors in spelling and naming (the English rural writer Harold Massingham is conflated with his editor father Henry, and newly christened 'Hugh' for instance); elegant and sympathetic when dealing at length with a real person such as Henry Williamson, yet often turgid and barbarous when bent on generalizing argument ('autarky' used for self-sufficiency). Yet there are clues to the author's motivation in the text. In her preface she talks elegiacally of a period of her life spent on a Herefordshire smallholding and of the lost "yeoman spirit". Later she applauds the English land-owning tradition, and in a revealing passage writes that "the nurture of the countryside is the first long-term aim of those who live in it, belong to it and wish to transfer it intact to their heirs. Whether a new rural proletariat, previously unemployed in the towns, inhabiting small, nationalised units of land, working farms organically, would offer such a nurture seems to me doubtful".

The familiar tones of 'Indignant, Hereford' can't be disguised any longer, and behind the stern intellectualism of Dr Bramwell's early chapters and the tirades of her coda, there lurks an old style patrician, who cannot bear to think of the old order, and its traditional flow of energy and authority, being disrupted. As she says in her concluding sentence, "The father of the movement is an utter rejection of all that is, and for at least three millennia all that was", a complaint that in the turbulent atmosphere of 1989 is a misplacement of blame of global proportions. □

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Now it can be told

Vera Rich

Uncovering Soviet Disasters: Exploring the limits of Glasnost. By James E. Oberg. Robert Hale: 1989. Pp.317. £14.95.

IN many respects, this book is a development of Oberg's earlier work *Red Star in Orbit* (Random House, 1981). Described as an "overview" of its subject, the "result of nearly a lifetime's interest and a full decade's active research", *Red Star in Orbit* was not a straightforward history of the Soviet space programme, but an exposé of Soviet secrecy and cover-ups, particularly as regards set-backs, accidents and loss of life or spacecraft. *Uncovering Soviet Disasters* is essentially a general application of the same approach.

With one notable exception (the loss of the prototype aircraft Maksim Gor'kii in 1935), Oberg confines his investigations to the period after 1950. He concentrates on mechanical failures and human error, aircraft and rail crashes, the loss of ships and submarines, nuclear accidents and — of course — space disasters. He ignores, however, natural disasters and near-disasters such as earthquakes and mudslides, even when there is evidence that human error or bad planning compounded the damage and death toll.

The chapter on "super-projects" confines itself to aircraft (the TU-144 Concorde), space (the aborted Moon and Mars programmes) and, somewhat strangely, the 600-cm Big Alt-Azimuth Telescope in the Caucasus, which is a disaster only in the sense of having failed to justify its enormous cost and advance publicity. But if the telescope finds a place in this book, why not the collapse of the Kodar tunnel on the Baikal-Amur Mainline railway a few months before it was due to open? Or the Kara-Bogaz-Gol barrage, which effectively destroyed the brine feedstocks of the Turkmenian chemical industry? Or the high dams of the Vakhsh valley, which, in that highly seismic area, can never be safely filled to capacity? Or the destruction of the Aral Sea by massive irrigation schemes? All these, in their time, were super-projects. And if loss of human life is the prime criterion for inclusion, why not a mention at least of the final disaster of the Stalin era, the stampede at the leader's funeral, due to poor policing, which crushed scores of people to death?

Furthermore, although Oberg subtitles his book "Exploring the limits of Glasnost", the revelations of *glasnost* have inevitably overtaken him. The book gives no cut-off date; however, the last entry in the appended "disaster chronology" is a train crash

on 7 August 1987. Since then, the Soviet press has carried several retrospective accounts of past accidents, from the Siberian nuclear explosion in 1958 to a 'Hillsborough'-type disaster at a football stadium eight years ago. Fall-out maps from Chernobyl have been published — likewise an account of the special "Chernobyl committee" set up in Estonia to care for national servicemen retained on clean-up operations long past the safety limit, all outdating Oberg's discussion. The Spitak earthquake, the Tajik landslide and the Urals train crash have all taken place in the eye of world publicity, making Oberg's work seem somewhat dated.

A more serious criticism may be applied to Oberg's methodology. *Uncovering Soviet Disasters* is a major improvement on *Red Star in Orbit* in that Oberg now cites his sources, which turn out to be limited. Citations from the Soviet press come almost entirely from the central newspapers *Pravda*, *Izvestiya* and *Krasnaya Zvezda*. One reference to *Pravda Ukrainy* comes via the FBIS-SU monitoring reports, but otherwise, Oberg seems to have made little if any use of local or republic papers. Nor are Soviet scientific and technical journals cited. Yet such sources are often a far more prompt and fruitful source of information on accidents than the central press, even in the era of *glasnost*. Thus the disastrous fire at the Leningrad Library of the Academy of Sciences in February 1988 was reported in *Leningradskaya Pravda* the following day, whereas the All-Union *Pravda* took up the story only several weeks later. And some Brezhnev-era disasters, like the 1973 mudslip which almost wiped out the city of Alma-Ata (the protective mud-traps were badly designed), were widely discussed at the time in engineering journals though not in the general media.

Oberg's assertions or implications that such an event was not reported in the contemporary Soviet media have to be taken with caution. Confidence is not restored by the occasional elementary error: he refers to the "Red Army" museum in Beijing instead of the "People's Liberation Army" ("Red Army" denotes the Soviet army; the Chinese do not use that term); or to the newspaper of the British Communist Party (in 1961) as the *Daily World* instead of the *Daily Worker*.

Perhaps more worrying is the case of the ship which Oberg calls the *Eshghabad*. One virtue of Oberg's previous book was his debunking of the many unsubstantiated Western rumours about unreported Soviet space disasters. The same scepticism is applied in the current work to the *Eshghabad*, reportedly lost with 270 passengers and crew in a storm on the Caspian on 14 May 1957. The Associated Press report of the sinking (from Iranian sources and, AP claimed, a confidential telegram from Baku) were never corro-



Spot the difference: although clearly visible in an earlier Soviet space photo (top), this "missing cosmonaut" was erased from later official editions of the same photo (bottom) — and replaced with fake shrubbery — after his expulsion from the space programme "due to an unpardonable act of arrogance".

borated further, Oberg says, although the disaster was incorporated into general Western reference books. Oberg doubts the story on the grounds that "The name 'Eshghabad' is not a proper transliteration of any Cyrillic spelling and it is strange for a Soviet ship to carry a Moslem name. The name may actually be somehow connected to the small village of Eshaqabad, southwest of Mashhad. Meanwhile, there is no *Eshghabad* (or any similar name) listed in the 1957 edition of *Lloyd's Register of Shipping*. . .". In fact it is no more strange for a Soviet vessel to carry this particular 'Moslem' name than it would be for a US vessel to bear a 'Hispanic-Catholic' name such as *San Francisco* or *Sacramento*. "Eshghabad" is simply an Iranian transcription of "Ashkhabad", capital of the Turkmen SSR. Oberg's rejection of the incident may well be valid, but his failure to recognize the name suggests that his general Soviet knowledge (as opposed to his specialized knowledge of the space programme), is limited.

The book is nevertheless fascinating. It presents an overall picture of a bumbling, hidebound bureaucracy, committed to scientific and technical progress, but unable to cope with and unwilling to admit to the inevitable set-backs. There was, for

example, no disaster rescue service apart from the army — a fact which, as Oberg rightly notes, has led certain events, such as the anthrax outbreak of 1979, to be wrongly given a military dimension by western commentators. (The establishment of a specialized civil disaster service was one of the first concerns of the new-style Supreme Soviet this June.)

Oberg's picture, which will not appeal to opponents of *glasnost*, records with pardonable pride the chagrin with which some Soviet space officials received his former revelations, in particular the celebrated pictures unearthed from newspaper files of trainee cosmonauts later airbrushed out of the group when they were dropped from the programme. To supporters of the Gorbachev reforms, however, Oberg's book will undoubtedly be yet further evidence of the need for *glasnost* — if only to forestall more Western exposés. Would the "flagship" journals of *glasnost* — *Moscow News* (say) or *Ogonek* — go so far as to review it favourably, or even reprint extracts not already outdated by Soviet revelations? In the current Soviet political climate, even this does not seem impossible. □

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Practically essential

Aksel A. Bothner-By

Nuclear Magnetic Resonance Spectroscopy, 2nd Edn. By Frank A. Bovey. Academic: 1988. Pp.653. \$49.95, £33.50.

ONE cannot be a chemist these days without knowing a good deal about nuclear magnetic resonance spectroscopy. Any current issue of a chemical or biochemical journal demonstrates that the technique is indispensable for establishing chemical structure, and today it is universally used for that purpose. Anybody who practises or wants to practise in this area has to become familiar with the theory and methods concerned, particularly as used for structural investigation, so that he may profit from this ubiquitous and powerful method.

In preparing the second edition of his book (the first appeared in 1969, since when NMR has evolved spectacularly), Dr Bovey set himself the task of making the methodology available to chemists who have not had a rigorous formal training in the quantum mechanics of particles with angular momentum. So there are

no hamiltonians, matrices (density or otherwise), commutators and the like to be found anywhere in the text.

Bovey has made a dedicated effort to present all the phenomena, interpretations, methods and empirical correlations in easily visualized terms. In many areas, this approach is gratifyingly successful — students, whether undergraduate, graduate or grey-haired, will find his book a valuable guide to this field, with extensive maps, charts and advice, all in readily understandable form.

But there is an inevitable price to be paid for treating an obstinately quantum mechanical phenomenon in classical and non-mathematical terms. Occasionally, the classical picture will not fit with what is observed in the laboratory, and the results of experiments then appear enigmatic, to say the least. The powerful two-dimensional methods developed in the past few years are unintelligible except in quantum mechanical terms, yet they yield structural data of immense importance; heteronuclear double quantum coherence is an example. Treatment of this family of experiments is sparse or absent from the book, and almost necessarily so. The qualitative description of NMR phenomena is, in places, dangerously loose and could leave the reader stranded on the wrong path.

Probably the strongest feature is Bovey's presentation of practical data: tables of shifts, coupling constants, nuclear properties, spectral patterns, and structure-spectra connections. These tables will certainly be valuable as guideposts in spectral interpretation, and will be of immediate use to those embarking on the NMR voyage of discovery. □

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other than that of reproduction. The phenomenon is shown by more than 200 species from over 40 families: and if there be "a purpose in liquidity", then surely there must also be a purpose in diadromy.

Although there are several reviews on the subject, there has been no one volume which brings the facts together. Robert McDowall has set out to make good the need and he does it well. The book is logically structured, passing from definitions to the occurrence of the three forms of diadromy among fish, and so on, to details of life histories and their ecological significance. The author reviews the problems in establishing diadromous populations where they do not occur naturally, both the commercial and recreational importance of diadromous species, and concerns over those that are endangered.

The approach to the subject is truly global; a vast amount of information has been abstracted from the literature (there are over 600 references, going up to 1987) and is presented to the reader in a series of intelligible figures and tables. McDowall suggests (p.119) that the direction of migrations — anadromous at high latitudes and feeding at sea, catadromous at low latitudes and feeding in freshwater — could be related to the observation that marine habitats are more productive at high latitudes and less so towards the tropics, whereas freshwater habitats have greatest productivity towards the tropics and less towards the poles. Right or wrong, this is a stimulating idea, and there are others.

I enjoyed the book and learned a great deal from it; it is unusually readable, and there is much here for the general reader.

Although semantic problems are dealt with carefully, it is disappointing to find no reference to John Kennedy's contributions to our understanding of the term migration. The index contains 700 itemized entries, which falls far short of that to be expected in a scholarly work of 300 pages: a string of page numbers is useless as a retrieval system for the serious student. There are also some curious errors, evidence of sloppy proof reading. A hyphen in Harden Jones is perhaps no more than one deserves, but to refer consistently to Professor A. D. Hasler as Haslar is inexcusable. □

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There and back again

F.R. Harden Jones

Diadromy in Fishes: Migrations Between Freshwater and Marine Environments. By Robert M. McDowall. Croom Helm (Chapman & Hall), London/Timber Press, Portland, Oregon: 1988. Pp.308. £30, \$47.95.

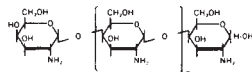
DIADROMY describes migrations in which fish move between the sea and freshwater. Anadromous fish (such as salmon) move from the sea to breed in freshwater; catadromous fish (eels) move from freshwater to breed in the sea; and amphidromous fish (whitebait and gobies) move between the two domains for pleasures

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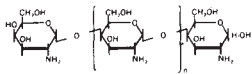
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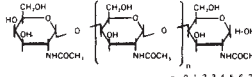
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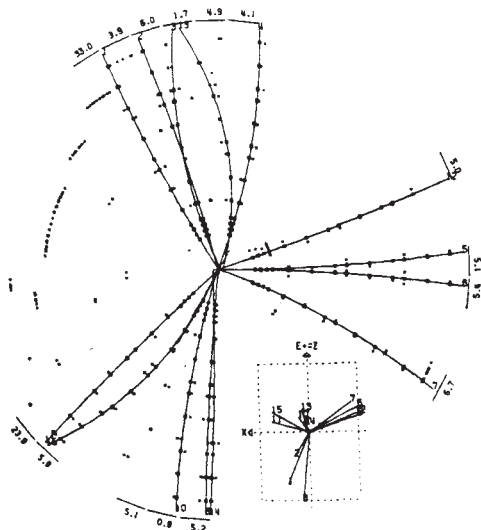


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The clean signature for particles produced at CERN's new Large Electron-Positron collider (LEP) should make possible the detection of exotic particles such as the Higgs boson and supersymmetric species. With precision measurements of important parameters, the Standard Model for elementary particles should be improved and tested.

In what follows, I summarize the specifications of the LEP accelerator and its four major detectors, outline the present Standard Model of elementary particle physics, including the reasons for expecting another quark, called the top (t), as well as some kind of Higgs boson (H). Much of the excitement about LEP is that it makes possible the design of various experiments to look for these and other particles, to count the number of neutrino species and to measure the parameters of the Standard Model much more precisely. But, naturally, one should not suppose that there is no physics beyond LEP.

At later stages, it is planned to increase the beam energy in steps to 90 or 100 GeV by 1994, at which stage the luminosity should reach $\sim 5 \times 10^{31} \text{ cm}^{-2} \text{ s}^{-1}$. This development will require an increase in the power of the radiofrequency accelerating cavities, which could alternatively be used at lower energies to increase the beam currents and hence the luminosity, perhaps above $10^{32} \text{ cm}^{-2} \text{ s}^{-1}$.



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Four experimental areas around the LEP ring have been equipped with large detectors called ALEPH, DELPHI, L3 and OPAL. Each is designed to detect all particles produced over a large fraction of the total 4π steradians of solid angle. But each detector has some distinctive features. ALEPH emphasizes highly granular three-dimensional tracking information. DELPHI is uniquely capable of distinguishing different species of strongly interacting particles. L3 pays special attention to the precise measurement of the momenta of electrons, muons and photons. OPAL uses well-established detection principles of proved reliability. But there is also provision for a few small specialized experiments; one of these, for example, will search for magnetic monopoles.

Many experiments have confirmed that the gauge group of the non-gravitational interaction is $SU(3)$ (for the strong forces) $\times SU(2) \times U(1)$ (for the 'unified electroweak' forces). There is no confirmed experiment contradicting this Standard Model, but it has not been fully verified and there are many important questions that are left open to be answered by LEP.

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observed are called up (u), down (d), strange (s), charm (c) and bottom (b). But there are good reasons to believe that quarks come in pairs, in which case at least one — the top (t) quark — remains to be found. Theoretically, because of the Adler–Bell–Jackiw anomalies, the Standard Model would not even be consistent at the quantum level if the fifth quark, bottom (b), were not paired¹. Experimentally, the neutralcurrent Z^0 couplings of the b quark measured² at lower-energy e^+e^- machines confirm that it is paired in an electroweak isospin doublet with another quark as yet unseen. Finally, the suppression of flavour-changing neutral couplings between bottom and strange or down quarks tells us³ that there must be a top quark. When combined with other electroweak data^{4,5}, this suggests that the mass of the top quark, m_t , lies between 50 and 200 GeV. Finding the top quark, or at least providing indirect information about its mass, will therefore be high on the agenda of LEP.

How many species of matter particle exist? Although the minimal version of the Standard Model contains just six quarks, three charged leptons (e, μ and τ) and three neutrinos — of which only two have been detected directly in the laboratory — there are no conclusive arguments to exclude the existence of more. Indeed (see above), quantum consistency and, in particular, the avoidance of anomalies, requires equal numbers of quark pairs and lepton pairs. The lepton pairs include massless (or almost massless) neutrinos, of which the first two are well-established while the existence of the third pair can be inferred from the decay of the W bosons and of the τ charged lepton. Because charged leptons and quarks could be very heavy, the most meaningful way of counting matter species may be to count the number of associated neutrino types (N_ν). Previous accelerator experiments, cosmology⁶ and the products of supernova 1987A (see refs 7,8) suggest that $N_\nu < 5$, but do not exclude⁹ $N_\nu = 4$. There are even models with additional weakly interacting neutral particles that would, in some experiments, mimic a fraction of a neutrino — so perhaps it may be that N_ν will turn out not to be an integer. Counting the apparent number of neutrino species will be an important task for LEP.

Are the different fundamental interactions unified? In the Standard Model, the strong SU(3) and electroweak SU(2) and U(1) coupling strengths are different and arbitrary, with the ratio of the latter fixing the electroweak mixing angle θ_w . If these interactions are unified in a simple non-Abelian group, the different gauge couplings are related. Simple versions of such grand unified theories (GUTs) predict (ref. 10) $\sin^2\theta_w = 0.20$ to 0.25, to be compared with the present experimental value

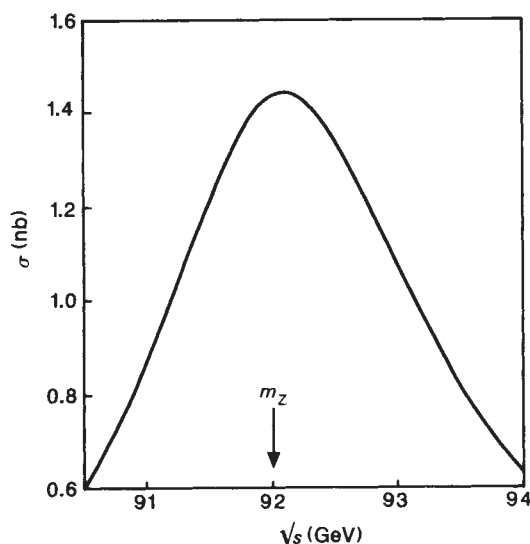


FIG. 2 The line-shape of the Z^0 peak in e^+e^- annihilation. The maximum is shifted 95 MeV above the mass of the Z^0 because of radiative corrections.

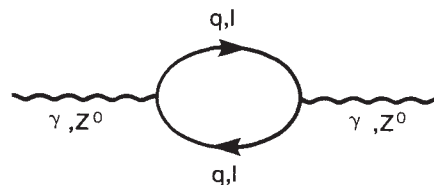


FIG. 3 The quantum effects due to virtual particles such as quarks and leptons, for example the top quark, polarize the vacuum and give radiative corrections to the mass of the Z^0 .

$\sin^2\theta_w = 0.228 \pm 0.004$ (refs 4,5). This constitutes circumstantial evidence for the GUT philosophy, but a more precise determination of $\sin^2\theta_w$ is necessary to discriminate between different GUT models. That, LEP will make possible.

What is the origin of the particle masses? In the Standard Model, these are generated by spontaneous gauge symmetry breaking through the Higgs mechanism, which entails that there should exist at least one physical Higgs scalar boson (H), whose couplings are proportional to particle masses, but whose own mass (m_H) is unknown¹¹. Nuclear physics experiments imply that $m_H \geq 18$ MeV, the absence of $K + \pi + H$ decays suggests that $m_H \geq 300$ MeV, and the absence of Higgs bosons in the decays of mesons containing b quarks may mean that $m_H \geq 4$ GeV. There are theoretical arguments that $m_H \leq 1$ TeV, but this leaves a wide mass range in which to find a Higgs boson. In my view, the discovery of a Higgs boson would be more important than that of another quark, or even the 1983 CERN discoveries of the $W^\pm$ and Z^0 bosons. There is still controversy about the particle mass-generation mechanism, and an elementary scalar boson would be a new type of particle signalling a new principle in fundamental physics.

Are there other new particles? In its second phase, LEP will be able to search for all possible electroweakly interacting particles with masses less than 100 GeV. My personal favourites are supersymmetric particles, which seem to be required for the theoretical consistency of models with elementary scalar bosons¹². Supersymmetric theories contain a complete zoo of particles that partner those known, but have spins differing by half a unit. Model calculations¹³ suggest that many of these 'sparticles' could be accessible to LEP.

The LEP experimental programme

For the first few years of LEP operation¹⁴, the focus will mainly be on the resonance peak of the Z^0 gauge boson, which has a mass of about 91 GeV. With the luminosity planned for LEP 1, one expects that each of the major detectors will record the production and decay of a Z^0 particle every few seconds, for totals of about 10^6 each per year. (By comparison, the SLC e^+e^- collider had produced about 120 Z^0 particles by the time of writing.) A million Z^0 s will permit precise measurements of the Z^0 properties — mass, decay rate and couplings to elementary matter particles — and searches for rare Z^0 decay modes — such as those into Higgs bosons, for example. LEP II will be able to produce thousands of pairs of $W^\pm$ bosons and check in detail the gauge theory predictions for their couplings, as well as extend the searches for new particles to larger masses. Within this general framework¹⁴, let us now examine in more detail some of the specific experiments that can be done at LEP, and see how they bear on the key physics questions aired above.

Precision measurements at the Z^0 peak. Previous experiments at CERN have measured^{4,5} the mass of the Z^0 to within about 1.5 GeV: experiments at LEP should certainly be able to pin it down to within 50 MeV, and even to within 10 MeV if beam polarization can be used to calibrate the beam energies. To measure m_Z with this precision, detailed knowledge of higher-order electromagnetic effects is needed. A task force at CERN during the last few months has been cross-checking calculations of these

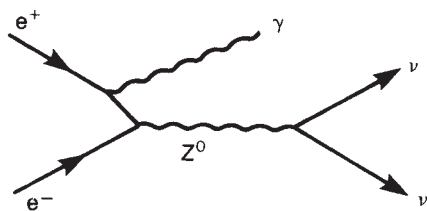


FIG. 4 Diagram of the neutrino-counting process $e^+e^- \rightarrow \gamma + (Z^0 \rightarrow \bar{\nu}\nu)$. The incoming electron and positron may radiate a photon before annihilating to make a Z^0 which then decays into a neutrino-antineutrino pair.

effects¹⁵, which shift the position of the Z^0 peak by about 95 MeV, as seen in Fig. 1. In the Standard Model, m_Z is determined by $\sin^2\theta_w$, but receives substantial radiative corrections of which the most important are due to vacuum polarization diagrams like those in Fig. 2. These include those due to virtual top quarks and are therefore sensitive to m_t . Indeed, the comparison between the values of $\sin^2\theta_w$ measured in low-energy experiments and that deduced from the present approximate measurement of m_Z already tells us that $m_t \approx 200$ GeV (refs 4,5,16). The future precision measurement of m_Z will fix m_t with an error of about 35 GeV (ref. 16), and measurements of Z^0 decay final states can fix m_t to within about 15 GeV, even if its mass puts the top quark beyond the reach of LEP. Radiative corrections are also sensitive to the unknown masses of other unseen particles, such as the Higgs boson. However, its effects are smaller, and longitudinally-polarized beams would be needed to obtain indirect information about m_H in this way¹⁷. Interpreted as precision measurements of $\sin^2\theta_w$, these precision measurements of the Z^0 mass and coupling will also distinguish between different GUTs, for example between those without supersymmetry, which typically predict $\sin^2\theta_w \approx 0.22$, and those with supersymmetry, which usually have $\sin^2\theta_w \approx 0.23$.

Neutrino counting. In the Standard Model^{14,15}, the total decay width of the Z^0 is predicted to be about 2.56 GeV. It can be measured with a precision of about 50 MeV at LEP, which is to be compared with the partial decay width of 170 MeV per neutrino species. Thus the LEP precision in the total decay width corresponds to $\Delta N_\nu = \pm 0.3$. However, if the Z^0 decay width turned out to be larger than expected we could not immediately assume that this was due to one (or more) extra neutrino species. Perhaps there were other unexpected Z^0 decay modes, and

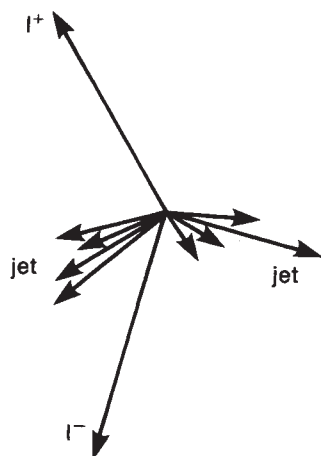


FIG. 5 Signature for $Z^0 \rightarrow H + l^+l^-$ decay. Coming away from the interaction point are two charged leptons and two jets of hadrons due to the decay of the Higgs boson.

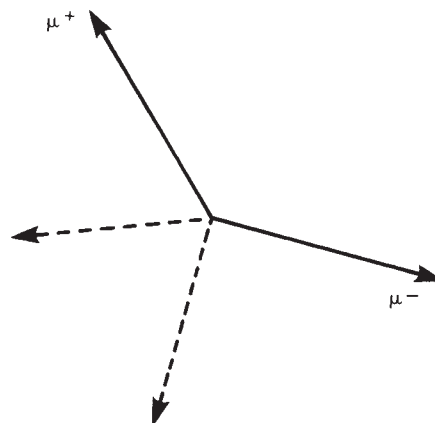


FIG. 6 Signature for $e^+e^- \rightarrow \text{smuon pair}$, followed by $\text{smuon} \rightarrow \mu + \text{missing energy}$. The only visible particles emerging from the annihilation point are a pair of charged leptons.

painstaking experimental work would be needed to disentangle them. An alternative neutrino-counting strategy¹⁸⁻²⁰ is to take data at an energy a few GeV above the Z^0 mass, and look for radiative Z^0 production — $e^+e^- \rightarrow Z^0 + \gamma$ — where only the photon (γ) with an energy of a few GeV is seen and the Z^0 decays into invisible weakly interacting particles as seen in Fig. 3. In the Standard Model, the cross-section for $e^+e^- \rightarrow \gamma + \text{nothing visible}$ at these energies is directly proportional to N_ν . In this way it should be possible to measure N_ν independently with a statistical precision $\Delta N_\nu = \pm 0.3$. As mentioned earlier, we should be open to the possibility that the apparent value of N_ν will not be an integer, because of Z^0 decays into other unobservable neutral particles. For example, in a minimal supersymmetric extension of the Standard Model one can have¹³ an apparent N_ν between 3.1 and 3.7.

Search for the Higgs boson. The first place in which the Higgs boson will be looked for at LEP is in Z^0 decay. The most promising channel seems to be $Z^0 \rightarrow H + \bar{L} + L$ decay²¹, where L is either a charged lepton or a neutrino, $\bar{L}$ its antiparticle. The branching ratio for $Z^0 \rightarrow H + e^+e^-$ or $\mu^+\mu^-$ varies between $\sim 10^{-4}$ if $m_H \leq 10$ GeV to $\sim 10^{-6}$ if $m_H \sim 50$ GeV. The dominant decays of the Higgs boson are expected to be into jets of hadrons, most probably containing b quarks. The signature for $Z^0 \rightarrow H + e^+e^-$ or $\mu^+\mu^-$ would therefore be a pair of leptons recoiling against a pair of jets with no missing energy, as seen in Fig. 4. Studies have indicated that this signature should be detectable above the backgrounds if $m_H < 50$ GeV. The signature for $Z^0 \rightarrow H + \bar{\nu}\nu$ would be just a pair of hadronic jets accompanied by missing energy. Picking this out requires good energy measurement (calorimetry), and so will not be easy, but the rate is about six times larger than for $Z^0 \rightarrow H + e^+e^-$ or $\mu^+\mu^-$.

At LEP II another interesting Higgs mechanism comes into play²², namely $e^+e^- \rightarrow Z^0 + H$, which has a rate large enough to be detectable in principle if $m_H \leq 90$ GeV. One could imagine looking for $Z^0 \rightarrow e^+e^-$, $\mu^+\mu^-$, $\bar{\nu}\nu$ or hadron decays. In the first two cases the signature would be very clean, but the rate relatively small. In the case of $Z^0 \rightarrow \bar{\nu}\nu$, the main signature would again be missing energy, which requires good calorimetry. Using these three channels, one should be able to find a Higgs boson if $m_H \leq 80$ GeV (ref. 23). More difficult is $H + Z^0 \rightarrow \text{hadrons}$, whose multi-jet final state would have large backgrounds from conventional strong interaction processes, and from $e^+e^- \rightarrow W^+W^-$ where both W particles decay hadronically.

Search for supersymmetric particles. In most supersymmetric models, the lightest 'sparticle' is electromagnetically neutral and has only weak interactions¹². It would therefore escape unseen from a detector in the same way as a neutrino. The Z^0 could

decay into pairs of these lightest sparticles, contributing to the apparent number of neutrino species N_ν as mentioned earlier. Heavier sparticles are expected to decay into the lightest sparticle, giving them a missing-energy signature. The lightest charged sparticles are expected to be sleptons, which would be pair-produced and therefore give the final-state signature of two acollinear charged leptons accompanied by missing energy as illustrated in Fig. 5. This signature is easy to pick out from the electromagnetic backgrounds, so charged sleptons will be seen at LEP I if they weigh 45 GeV, and at LEP II if they weigh ≤ 80 GeV (ref. 23). Other sparticles which may be accessible to LEP include the spartners of the $W^\pm$ bosons.

There are no definite predictions for the sparticle masses, but a general belief that for reasons of theoretical consistency¹² they should weigh ≤ 1 TeV. However, it turns out that the minimal supersymmetric extension of the Standard Model is subject to so many phenomenological and theoretical constraints that its unknown parameters are severely restricted¹³. One possible constraint is that the relic density of the lightest sparticles left over from the Big Bang not be too large, or even that they could constitute the dark matter favoured by astrophysicists. This latter requirement imposes upper limits on the sparticle masses, suggesting that the charged sleptons weigh ≤ 70 GeV (ref. 13), in which case they would be seen at LEP. This model also predicts that one or more Higgs bosons should be visible at LEP, as well as the t quark. There could be a 'scornucopia' at LEP which also casts light on the dark matter of the Universe!

Other possible new physics. Supersymmetry is just one of many ideas for going beyond the Standard Model. Alternatively, perhaps the Higgs boson is composite, rather than elementary, in which case the technical motivation for supersymmetry would disappear. Realistic 'technicolour' models with composite Higgs bosons predict many detectable charged and neutral particles in the LEP range¹⁴. Or perhaps quarks and leptons are composite — just another layer of the onion? In this case one could expect there to be excited quark and lepton states, the known quarks and leptons should have form factors, and there might be additional 'contact' interactions between them. Present-day e^+e^- machines give the most stringent lower limits on such phenomena, and LEP will probe for compositeness at distances down to about 10^{-18} cm, further than any other existing accelerator²³. Or perhaps the $W^\pm$ and Z^0 bosons are composite, and this is why they are massive? In this case, by analogy with the known composite vector mesons made out of quarks, one would expect more W' and Z' bosons to exist with larger masses. Their indirect effects — additional charged and neutral current interactions — could be seen at LEP II.

Outlook

Probably the greatest discovery at LEP will be none of the above, but a truly unexpected discovery. The beauty of LEP is that e^+e^- collisions are very democratic, producing all new particles with similar probabilities, unlike hadron-hadron collisions which mainly produce strongly interacting particles. Moreover, e^+e^- collisions are very clean, with no hadronic debris to mask subtle signatures such as those for the Higgs boson. Although the range of particle masses up to about 100 GeV has already been explored by $p\bar{p}$ colliders at CERN and more recently Fermilab, any number of new phenomena could

still be lurking undetected in this mass range. Hadron-hadron colliders have been able to detect the $W^\pm$ and Z^0 — electro-weakly interacting particles with dramatically clean signatures. However, they have not even been able to rule out new strongly interacting particles with masses ≤ 100 GeV, let alone hard-to-find electroweakly interacting particles such as the Higgs boson. LEP is the first accelerator with a fighting chance of finding one — but no promises since we do not know the mass of the Higgs boson. LEP will be able to make whatever physics may chance to be in the energy range up to 100 GeV, a *terra* still largely *incognita*.

In addition to being an accelerator, LEP is real estate, the largest accelerator tunnel ever constructed. There is space inside it for other rings of accelerator magnets, should physics demand it. An option under active consideration for the late 1990s after LEP II is to install a double ring of high-field superconducting magnets for proton beams with energies up to 8 TeV each. Known²⁴ as the Large Hadron Collider (LHC), this project could provide $p\bar{p}$ collisions at a centre-of-mass energy of 16 TeV, or collide with LEP to give e^-p collisions at about 1.5 TeV in the centre-of-mass (see the Table). CERN's sources of heavy ions

Present and future particle accelerators				
		Location	Centre-of-mass energy	Luminosity ($\text{cm}^{-2} \text{s}^{-1}$)
e^+e^-	TRISTAN	Japan	60 GeV	10^{31}
	SLC	USA	100 GeV	10^{28}
	LEP I	Europe	110 GeV	$10^{31}/10^{32}?$
	LEP II		190 GeV	5×10^{31}
ep	HERA	W. Ger.	314 GeV	10^{31}
	LHC ?	Europe	1.5 TeV	10^{32}
$p\bar{p}$				
	CERN SPS	Europe	0.63 TeV	2×10^{30}
	FNAL	USA	1.8 TeV	10^{30}
pp	UNK ?	USSR	6 TeV	10^{31}
pp	LHC ?	Europe	16 TeV	$10^{33}/5 \times 10^{34}$
	SSC ?	USA	40 TeV	10^{33}
Heavy ions				
	LHC ?	Europe	82 TeV	10^{28}

could also be used to provide heavy-ion collisions in the LHC. With a $p\bar{p}$ luminosity of $10^{33} \text{ cm}^{-2} \text{ s}^{-1}$ to a few times $10^{34} \text{ cm}^{-2} \text{ s}^{-1}$, the LHC project could make a preliminary exploration of physics in the TeV range, analogous to that made of the 100-GeV energy range by the previous CERN $p\bar{p}$ collider. (For comparison, the US Superconducting Super Collider would have a centre-of-mass energy of 40 TeV and a design luminosity of $10^{33} \text{ cm}^{-2} \text{ s}^{-1}$. The LHC could do much of the same physics at a fraction of the cost, as well as having unique e^-p and heavy-ion options.) If there are any more gauge bosons, heavy quarks or strongly interacting supersymmetric particles, the LHC could find them²⁴. It would also be able to find the Higgs boson if it weighed between 200 GeV and 1 TeV. Thus the useful life of LEP could be extended well into the next century. $\square$

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- Bouchiat, C. Iliopoulos, J. & Meyer, P. *Phys. Lett.* **38B**, 519 (1972).
- Bartel, W. *et al. Phys. Lett.* **146B**, 437 (1984).
- Kane, G.L. & Peskin, M. *Nucl. Phys.* **B195**, 29 (1982).
- Amaldi, U. *et al. Phys. Rev.* **D36**, 1385 (1987).
- Costa, G. *et al. Nucl. Phys.* **B297**, 244 (1988).
- Yang, J. *et al. Astrophys. J.* **281**, 493 (1984).
- Ellis, J. & Olive, K. *Phys. Lett.* **B193**, 239 (1987).
- Schramm, D. *Comments Nucl. Part. Phys.* **17**, 239 (1987).
- Denegri, D. Sadoulet, B. & Spiro, M. *CERN Preprint EP/89-72* (1989).
- Georgi, H., Quinn, H. & Weinberg, S. *Phys. Rev. Lett.* **33**, 451 (1974).
- Cahn, R.N. *Lawrence Berkeley Laboratory Preprint LBL-26433* (1988).
- Haber, H. & Kane, G. *Physics Rep.* **117C**, 75 (1985).

- Ellis, J. & Zwirner, F. *CERN Preprint TH. 5460/89* (1989).
- Ellis, J. & Peccei, R.D. (eds) *CERN Report 86-02* (1986).
- Altarelli, G. & Kleiss, R. (eds) *Z Physics at LEP* (in preparation).
- Ellis, J. & Fogli, G.L. *CERN Preprint TH. 5457/89* (1989).
- Alexander, G. *et al. (eds) CERN Report 88-06* (1988).
- Dolgov, A., Okun, L.B. & Zakharov, V.I. *Nucl. Phys.* **B41**, 197 (1972).
- Ma, E. & Okada, J. *Phys. Rev. Lett.* **41**, 287 (1978).
- Gaemers, K.J.F., Gastmans, R. & Renard, F.M. *Phys. Rev.* **D19**, 1605 (1979).
- Bjorken, J.D. *Proc. 1976 SLAC Summer Inst. Particle Phys.* (SLAC-198, 1977).
- Lee, B.W. Quigg, C. & Thacker, H. *Phys. Rev.* **D16**, 1519 (1977).
- Böhm, A. & Hoogland, W. (eds) *CERN Report 87-06* (1987).
- Mulvey, J. (ed) *CERN Report 87-07* (1987).

The anthropic significance of the existence of an excited state of ^{12}C

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Were it not for the presence of an excited state of ^{12}C at 7.6 MeV, at the endpoint of the triple-alpha reaction, it would be difficult for stars to manufacture carbon and heavier elements. Calculations using modified triple-alpha rates test the sensitivity of stellar nucleosynthesis to the exact position of this excited state, and allow an empirical assessment of its importance in the anthropic principle in cosmology.

THERE has been much discussion of the 'coincidences' associated with the nuclear resonance levels of carbon and oxygen, which are essential for biological evolution, in relation to the anthropic principle¹. The significance of such discussions lies in the fact that they deal with physical quantities within the realm of experimentally verifiable physics (rather than, say, with the yet uncertain physics of the early Universe). In particular, considerable attention has been focused on the 0^+ excited nuclear state of ^{12}C , at 7.644 MeV, which lies just above the energy of ^8Be plus an α -particle. In a truly remarkable prediction, Hoyle concluded from the observed cosmic-abundance ratios of $^{16}\text{O} : ^{12}\text{C} : ^4\text{He}$ that such a resonant level should exist. This has been confirmed subsequently by experiment²⁻⁴.

As is consistent with the anthropic principle^{1,5}, the energy of the resonant level of ^{12}C is required to have the value it does, to ensure carbon production and the consequent development of carbon-based life. In a broader context, the location of the resonant level of carbon is the middle of three 'coincidences'. The first of these is the fact that the decay lifetime of ^8Be is about four orders of magnitude longer than the time required for two α -particles to scatter past one another in a non-resonant manner. This ensures the build-up of a small concentration of ^8Be , which can come into equilibrium⁶, thus allowing its coexistence with ^4He . The third 'coincidence' is the fact that the energy level of ^{16}O at 7.1187 MeV is non-resonant, being below the combined energy of $^{12}\text{C} + \alpha$ (at 7.1616 MeV). This ensures that a significant fraction of the ^{12}C created will not be destroyed by α -particle capture.

In this exploratory work, we examine the question of how hypothetical changes in the location of the carbon 0^+ nuclear level might affect nucleosynthesis, carbon production, and its mixing into the interstellar medium (ISM). We note that a change in the nuclear energy levels should really be a consequence of changes in strengths of the fundamental interactions (strong, electromagnetic and so on). Such fundamental changes would affect not only the 0^+ level of ^{12}C but also many other nuclear energy levels. Indeed, these changes might result in a completely different structure and evolution of our Universe, and hence the incorporation of all of these changes is both beyond the capability of present-day theories of nuclear physics and certainly beyond the scope of this paper. We therefore limit ourselves to considerations of the anthropic argument that are related to changes in carbon production resulting from changes in the location of the 7.644-MeV level of ^{12}C . Calculations have been performed both for core triple-alpha burning and for shell

helium burning in a thermally pulsing, asymptotic-giant-branch (AGB) star.

Core He burning in massive stars

One possible site of carbon production in stellar interiors is the core, and later the adjoining layers, of massive stars. Although in later phases of the evolution of stellar cores most of the products of helium burning will be processed to even heavier nuclei, there always remains carbon- and oxygen-rich matter that will be returned to the interstellar medium in the final supernova explosion. At the present age of the Galaxy, supernovae are probably not the main source of carbon production; rather, carbon production is expected to occur in thermal pulses in the shells of AGB stars. However, in the very early stages of galactic history, they were the only source, because less massive stars had not yet evolved far enough to return carbon to the ISM. It seems most appropriate, in the context of the anthropic principle, to investigate all possible sites of carbon production.

We have evolved a $20\text{-}M_{\odot}$ star through the completion of core helium burning, using a modified 0^+ level of ^{12}C , to identify the effects both on the amount of carbon produced and on the evolution. We evolve essentially the same model star as was considered by Truran and Weiss⁷ in the context of supernova 1987A, using the same input physics and evolution code as described therein.

The only significant difference from the calculation of ref. 7 is the treatment of the triple-alpha process. In the code, the formula for the energy generation rate by this process is⁸

$$\epsilon_{3\alpha} = \rho^2 Y^3 T_9^{-3} \exp(26.68 - 42.94/T_9) + \rho^2 Y^3 T_9^{-3} \exp(29.90 - 274.33/T_9) \quad (1)$$

where the first term arises from the 7.644-MeV resonance and the second from the 9.64-MeV resonance. The latter state does not contribute significantly for ordinary stellar burning conditions, and is usually neglected. In equation (1), ϵ is the energy generation rate in $\text{erg g}^{-1} \text{s}^{-1}$, ρ the density in g cm^{-3} , Y the mass fraction of helium, and T_9 is the temperature in units of 10^9 K . The term $-42.94/T_9$ in the argument of the first exponential function arises from $-\Delta E/kT$, where k is the Boltzmann constant, and ΔE is the energy difference between a carbon nucleus in the 0^+ state and three α -particles (see, for example, ref. 9), which amounts to 370 keV. The equivalent term in the second exponential function is that for the 3^- state, where the energy difference is 2,370 keV. It can be seen immediately that if the lower resonance state did not exist, the temperature would have to be 6.5 times higher to make the higher resonance comparably effective. However, at such a temperature, which would be close to 10^9 K (as compared to $\geq 10^8 \text{ K}$ in standard helium-burning cores), a greater variety of nuclear reactions involving heavier nuclei can occur, and such a star would evolve very differently from ordinary stars.

For the first example, we investigated the effect that would result from the total non-existence of the 0^+ resonance. We considered a star of typical population I composition: $X = 0.739$, $Y = 0.240$ and $Z = 0.021$ where X is the mass fraction of hydrogen and Z is the mass fraction of all elements except hydrogen and helium. Thus, the initial carbon abundance by

mass was ~ 0.005 and that of oxygen was ~ 0.010 . This would of course be inconsistent with a conclusion that carbon cannot be created in a stellar environment under these circumstances; however, the initial abundance is much lower than that produced during ordinary helium burning, and our calculations confirmed that the results do not depend on the initial amount of carbon.

The evolution proceeded as follows. After the star completed its hydrogen-burning phase, the core contracted and heated up in the usual manner. In a standard comparison star, with the 0^+ level included at its normal energy, helium burning was initiated at $\sim 1.5 \times 10^8$ K, with the $3\alpha \rightarrow {}^{12}\text{C}$ reaction dominating over the competing ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction, thereby converting helium mainly into carbon. (Subsequently, when the helium abundance had decreased and the temperature increased, the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction started to dominate and the carbon abundance decreased from a maximum of $\sim 50\%$ to $\sim 10\text{--}20\%$.) In our present model, the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction alone proceeded at about the same temperature, consuming all the initial carbon within less than 10,000 years. At that point, the star had effectively corrected the initial inconsistency in carbon abundance. The next reaction to be considered is then ${}^{16}\text{O}(\alpha, \gamma){}^{20}\text{Ne}$. The core contracted further until it reached a temperature of 2.8×10^8 K, at which point oxygen was burned. The core then consisted of helium and heavier nuclei, with no trace of carbon or oxygen; the temperature at this stage was already as high as it is in normal stars at the end of helium burning.

We did not follow the evolution much further, as it was clear that the core would now contract rather quickly to attain a temperature of $\sim 10^9$ K. At this temperature a significantly more complex model of nucleosynthesis would be necessary to follow all of the processes taking place. We can conclude that if the 0^+ level did not exist at all, even primordial carbon would be destroyed in the cores of massive stars. Even at temperatures sufficiently high to ensure that the triple-alpha reaction would proceed through the 9.64-MeV level, any ${}^{12}\text{C}$ formed would be processed up an α -particle chain to intermediate and heavy nuclei.

For our next example, we included the 0^+ resonance, but at an energy difference with respect to ${}^8\text{Be} + \alpha$ of 860 keV, as opposed to the true difference of 370 keV. The evolution was similar to that described above except that now the triple-alpha reaction was able to proceed at the same temperature as the ${}^{16}\text{O}(\alpha, \gamma){}^{20}\text{Ne}$ reaction, in such a way that it formed carbon, which was converted immediately into oxygen and then into neon. Ultimately, the core matter was again carbon- and oxygen-free, but showed a slightly smaller helium abundance.

We then reduced the 0^+ level further, to an energy difference of 647 keV. The model now exhibited a distinct ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ burning phase, with some contribution from the triple-alpha process. Carbon was again destroyed almost completely except for a trace mass abundance of 10^{-4} , but the oxygen abundance increased to 0.02. This means that, in addition to the initial carbon abundance of 0.005, the same mass fraction of helium was burned to ${}^{12}\text{C}$ and beyond. If the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction alone were to operate, the decrease in helium mass fraction would be only one third of that of carbon. Indeed, the energy liberated by the triple-alpha process was twice as large as that from the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction and nine-times larger than that from the ${}^{16}\text{O}(\alpha, \gamma){}^{20}\text{Ne}$ reaction. This confirms the fact that the triple-alpha process is indeed operating. The detailed behaviour seems to be somewhat dependent on the initial abundances of C and O. The final state of the model reflected the fact that helium eventually is converted into oxygen by the chain of reactions $3\alpha \rightarrow {}^{12}\text{C} \rightarrow {}^{16}\text{O}$, with the equilibrium value of carbon being extremely close to zero. We estimate that the model would ultimately have burned oxygen via the ${}^{16}\text{O}(\alpha, \gamma){}^{20}\text{Ne}$ reaction, but that some oxygen could survive the helium-burning phase. We did not follow the evolution after carbon had reached its equilibrium value.

In a further experiment, we used a 0^+ energy difference of

430 keV. In this case the 3α process dominated at the beginning of helium burning, and carbon abundance increased from its primordial value to 9.5% by mass. This is an increase by a factor of almost 20. However, when this abundance level was reached in the core, the central temperature was high enough (2×10^8 K) for the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction to become dominant, and carbon decreased again, until finally all helium and carbon in the convective core (10% of the stellar mass) had been converted into oxygen. Thus, in its further evolution, the core of the star (in which all subsequent nuclear reactions up to the final collapse will occur) would be carbon-free. However, whatever the final structure and composition of the core might be, it will always be overlain by a partially burned helium shell. At the end of helium burning, this shell contained $\sim 6\%$ of the stellar mass (see Fig. 1). Within this shell, carbon is present with a maximum abundance by mass of $\sim 10\%$ and an average abundance of half this value. Thus, at the time of the supernova explosion, we estimate that $5 \times 10^{-4} M_\odot$ of fresh, newly created carbon would be ejected into the ISM. This value is very low compared with the amount of carbon produced in a real $20\text{-}M_\odot$ star, but our result nevertheless indicates that a shift of the energy difference from 370 to 430 keV still allows carbon production.

Thus it is evident that carbon production in the cores of massive stars and its dependence on the location of the 0^+ level involves very similar considerations to those influencing the amount of carbon remaining after helium burning as a function of the exact ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ rate. Indeed, as there exist two competing processes (one carbon-producing and the other carbon-consuming), the final carbon abundance depends on the relative efficacy of these processes. When it was determined several years ago that the ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ reaction rate had to be increased¹⁰, thereby making this process more effective with respect to $3\alpha \rightarrow {}^{12}\text{C}$, the carbon abundance produced in stellar models was found to decrease accordingly by roughly a factor of two to three. (Note that recent experiments, however, indicate a lower ${}^{12}\text{C}(\alpha, \gamma){}^{16}\text{O}$ rate (W. Fowler, personal communication).) In our numerical experiments, we basically achieve the same end by making the $3\alpha \rightarrow {}^{12}\text{C}$ reaction less effective. The features of the helium-burning phase are thus both easy to understand and rather predictable.

In all these calculations we have increased the energy difference between the $({}^8\text{Be} + {}^4\text{He})$ and 0^+ states. Making an anthropic statement that requires the 0^+ resonance to be at its observed level, however, raises also the question of how carbon

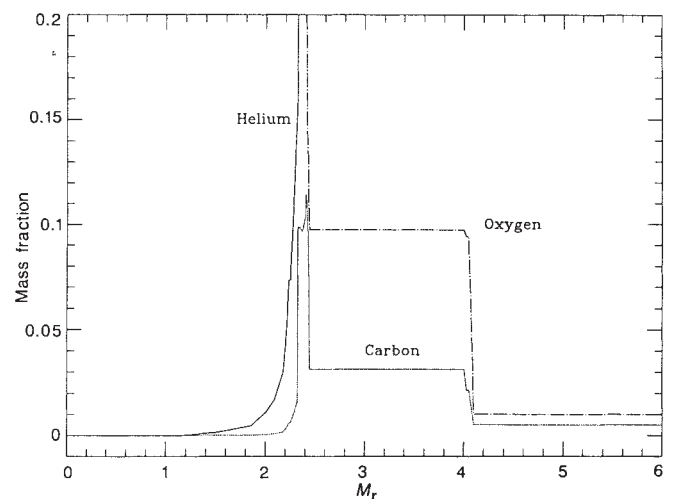


FIG. 1 The mass fractions of helium, carbon and oxygen in the helium core of a $20\text{-}M_\odot$ model star at the end of central helium burning, as a function of the internal mass fraction M_r , for the case in which the energy of the 0^+ level of ${}^{12}\text{C}$ is taken to be 430 keV above the sum of the masses ${}^8\text{Be} + \alpha$. Note that the ordinate extends only to 0.2.

production is affected in the case of a smaller energy difference. From the discussion above, the answer can be predicted easily: more carbon would be produced during helium burning. To answer the question more quantitatively, we performed another experiment in which the 0^+ level was lowered by 60 keV. The result is surprising. At the end of the core helium-burning phase, carbon had a mass fraction of 0.794, which is roughly four times higher than normal. Comparing this with the result of increasing the level by 60 keV, it appears that carbon production is not strongly favoured by nature, because a small reduction in the energy difference would lead to a relatively much greater increase in carbon abundance than the respective decrease that results from an equivalent increase in the energy difference. In terms of the anthropic principle, one might say that "things could be worse, but they could easily be much better."

We can make some remarks on the implications of our numerical experiments for the global behaviour of the models. The higher the energy of the 0^+ level, the more the core contracted, and in consequence, the more the envelope expanded. In the first model (no 0^+ level), for example, the star reached the red-giant branch before carbon had been burned, whereas in the final model it was still at a temperature of 10,000 K at the end of helium burning. When the energy of the resonance is decreased, the convective core increases in mass. This is all a result of the fact that 'blocking' the triple-alpha process leads to a decreased energy output of the core.

Shell He burning in AGB stars

Observations indicate that the surfaces of AGB stars become contaminated with carbon, and it is believed that these AGB stars are a major source of carbon (as well as many other heavy elements) in galaxies. Theoretical investigations have demonstrated that the appearance of carbon at the stellar surface is a consequence of a thermal instability occurring in the helium-burning shell which surrounds the degenerate carbon-oxygen (C-O) core¹¹. The carbon-enhanced surface material is introduced into the ISM by relatively rapid mass loss (timescales $\tau_{\text{mass loss}} \ll \tau_{\text{nuclear}}$) which occurs as a consequence of a pulsational instability in the expanded red-supergiant envelope^{12,13}. We have studied how a change in the energy of the carbon 7.644-MeV level can affect both the energetics of the AGB thermal instability and the nucleosynthesis that occurs during the subsequent thermal runaway.

We consider a $5-M_{\odot}$ AGB star with a $0.96-M_{\odot}$ C-O core, and we assume that this star initially had a solar metallicity. This assumption is again potentially inconsistent with the carbon energy level being different from its true value, but we suggest that this should not influence our conclusions significantly. The AGB star has been modelled through a thermal pulse using the evolutionary code described by Iben¹⁴. Three sequences have been calculated: in sequences I (the standard sequence), II and III we have assumed that the 0^+ level of carbon is 370, 430 and 647 keV, respectively, above the $^8\text{Be} + \alpha$ energy.

This AGB star will spend $\sim 90\%$ of its lifetime burning hydrogen into helium in a thin shell. The hydrogen-burning shell deposits this helium (as well as some nitrogen, via the CNO cycle) into the helium-rich (or helium- and carbon-rich) region that lies above the degenerate C-O core. When a sufficient quantity of helium accumulates in this region, triple-alpha burning commences in a runaway fashion, and in the standard sequence most of the runaway energy is supplied by the triple-alpha reaction. A convective shell forms below the hydrogen-burning region, and this shell provides fuel (helium, carbon and nitrogen in the standard sequence) for the runaway occurring at the base of the shell. When fresh ^{14}N is introduced into the convective shell, the luminosity from the $^{14}\text{N}(\alpha, \gamma)^{18}\text{O}$ reaction will temporarily exceed that from the $3\alpha \rightarrow ^{12}\text{C}$ reaction.

The byproducts of the runaway (carbon, oxygen and neon) are distributed throughout the convective layer during this phase. When the convective shell is at its maximum extent (in

the stellar mass coordinate), only one ^{12}C nucleus in the shell is destroyed (by α -particle capture) for every 50 that are created (by the triple-alpha process). The composition of the shell is then 83% helium and 15% carbon (by mass). This carbon at the upper edge of the convective shell is eventually mixed through the base of a convective envelope to the stellar surface, and subsequently into the ISM.

In the models of sequence II, the evolution of the thermal pulse is similar to that in the standard sequence. The reduction in the triple-alpha reaction rate causes the pulse to develop somewhat more slowly, and hence makes the thermal instability ignite helium under conditions of higher pressure and density (and at a smaller radial coordinate) compared with the standard sequence. Consequently, a more massive convective shell is produced, and a higher shell temperature allows both the $3\alpha \rightarrow ^{12}\text{C}$ and $^{12}\text{C}(\alpha, \gamma)^{16}\text{O}$ reactions to proceed more rapidly. In this case, when the convective shell reaches its maximum extent, ^{12}C is created approximately 20-times faster than it is destroyed, and carbon and helium are still the major constituents of the convective shell. In fact, because the convective shell itself is more massive, the amount of carbon mixed to the stellar surface during a later phase may be larger in this sequence than in the standard sequence.

The situation is radically different for sequence III. For sequences I and II, the development of the thermal pulse depends ultimately on the ignition of the $3\alpha \rightarrow ^{12}\text{C}$ reaction in the helium-rich region overlaying the degenerate C-O core. In sequence III, however, the luminosity from the triple-alpha reaction is orders of magnitude smaller than the luminosity from α -particle capture on ^{12}C , ^{14}N or ^{18}O . The calculations show that any ^{12}C that is produced during the thermal pulse is quickly converted to ^{16}O . We find, therefore, that carbon production by AGB-star thermal pulses does not occur when the 0^+ level is moved as high as 647 keV above the $^8\text{Be} + \alpha$ energy. It should be noted that the thermal pulse in sequence III was not modelled to completion, because of numerical difficulties arising in the models from the interplay between α -particle capture on ^{14}N and ^{18}O (which powers the runaway) and the rate at which unprocessed ^{14}N is introduced into the convective shell.

We note that the production of carbon also can be affected by the rate at which ^{12}C is destroyed in AGB-star models. It has been shown, for example, that the abundance ratio $^{16}\text{O}/^{12}\text{C}$ at the surface of a model star increases as the rate of the $^{12}\text{C}(\alpha, \gamma)^{16}\text{O}$ reaction increases¹⁵. Furthermore, we have considered the effect of an increase in the energy of the 0^+ level of ^{12}C on AGB models, but not the effect of a decrease. The latter would result in an enhanced rate of ^{12}C production, and would have to be evolved through (at least) several successive pulses to properly determine the extent to which ^{12}C becomes enhanced (above the 'standard' 15% mass abundance) in the AGB interior. Decreasing the 0^+ energy level would also make each thermal pulse weaker than in the standard model, and it is unclear whether these weaker pulses could provide the energy required to cause ^{12}C -enhanced material to be transported from the interior to the surface of a model AGB star.

Conclusions

On the basis of a series of numerical experiments, we have determined that a 60-keV increase in the location of the 0^+ level of carbon does not significantly alter the level of carbon production in stellar environments. Although the amount of carbon produced (relative to oxygen) is reduced, both in core burning and in shell burning, the strength of the thermal pulse in AGB stars is increased. This may increase the amount of interior (processed) material that is ultimately mixed to the stellar surface and thereby lost to the ISM. Also, a decrease in the location of the 0^+ level by the same amount leads to significantly greater carbon production. If the nuclear energy level is increased by 277 keV or more, the results of nuclear burning are radically different and very little carbon is produced.

The implications of these results for evaluating the anthropic 'coincidence' on which our existence seems to rely are not entirely free from subjective feelings. Whether or not one must conclude that the 0^+ level has to be exactly where it is depends on whether we should regard a change of 60 keV as small or large. We believe that, although it is true that 60 keV is small compared with the spacing between the 7.644-MeV level and

the next higher level at 9.64 MeV, this shift clearly represents a significant fraction of the energy difference between the 0^+ level and the energy of $^8\text{Be} + \alpha$. Thus, we believe that at least some formulations of the strong anthropic principle, which is based on the necessity of having the 0^+ level exactly where it is, is weakened significantly by our results. $\square$

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1. Barrow, J. D. & Tipler, F. J. in *The Anthropic Cosmological Principle* 250–255 (Clarendon Press, Oxford, 1986).
2. Hoyle, F. *Astrophys. J. Suppl.* **1**, 121–146 (1954).
3. Dunbar, D. N. F., Pixley, R. E., Wenzel, W. A. & Whaling, W. *Phys. Rev.* **92**, 649–650 (1953).
4. Hoyle, F., Dunbar, D. N. F., Wenzel, W. A. & Whaling, W. *Phys. Rev.* **92**, 1095 (1953).
5. Carter, B. in *Confrontation of Cosmological Theories with Observation* (ed. Longair, M. S.) 291–298 (Reidel, Dordrecht, 1974).
6. Salpeter, E. E. *Phys. Rev.* **107**, 516–525 (1957).
7. Truran, J. W. & Weiss, A. in *Supernova 1987A in the Large Magellanic Cloud* (eds Kafatos, M. & Michalitsianos, A.) 313–322 (Cambridge University Press, 1988).
8. Fowler, W. A., Caughian, G. R. & Zimmerman, B. A. *Rev. Astr. Astrophys.* **5**, 525–570 (1967).

9. Clayton, D. D. *Principles of Stellar Evolution and Nucleosynthesis* 414 (McGraw-Hill, New York, 1968).
10. Rolfs, C. in *Nucleosynthesis* (eds Arnett, W. D. & Truran, J. W.) 15–27 (Chicago University Press, 1985).
11. Iben, I. Jr & Truran, J. W. *Astrophys. J.* **220**, 980–995 (1978).
12. Wood, P. R. in *Physical Processes in Red Giants* (eds Iben, I. Jr & Renzini, A.) 205–224 (Reidel, Dordrecht, 1981).
13. Willson, L. A. in *Late Stages of Stellar Evolution* (eds Kwok, S. & Pottasch, S. R.) 253–268 (Reidel, Dordrecht, 1987).
14. Iben, I. Jr *Astrophys. J.* **196**, 525–548 (1975).
15. Becker, S. A. & Iben, I. Jr *Astrophys. J.* **237**, 111–129 (1980).

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Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements

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Nanometre-level analyses of the movements of membrane glycoproteins tagged with gold particles demonstrate that diffusing particles are not under the influence of a lipid flow, although a subset of particles which appear attached to the cytoskeleton are moving rearward.

DIRECTED motions of membrane proteins occur in antibody capping, cell migration and the assembly of the extracellular matrix, processes which are important in the immune response and organogenesis. It has been proposed that the driving force for these motions might be membrane flow^{1,2} or cytoskeletal movements³. Analysis of glycoprotein movements at the molecular level can differentiate between these mechanisms, because the velocity and path of a glycoprotein provides a distinctive fingerprint of the molecular mechanism of its movement.

The membrane-flow model suggests that membrane lipid flows back from the leading edge of the cell to sites of endocytosis and is then recycled to the front of the cell again^{1,2}. Membrane glycoproteins are supposed to float freely in the lipid bilayer, whereas large particles or aggregates of crosslinked glycoproteins, which diffuse slowly, will be swept rearward by the flow¹. The rapid diffusion of small particles or uncrosslinked molecules counters the flow and prevents the development of a substantial concentration gradient. Nevertheless, the effect of lipid flow even on rapidly diffusing particles should be detectable in sufficiently precise measurements.

Other models propose that glycoproteins are driven by interaction with rearward-moving cytoskeletal structures^{3–5}. Recent studies have shown that the actin cytoskeleton and drifting particles on the cell surface move rearward at the same velocity^{3–6}. Crosslinked glycoproteins strongly bound to the cyto-

skeleton would diffuse slowly and be dragged rearward. Particles free of cytoskeletal interactions would diffuse more rapidly without rearward drift. Detection of a rearward drift of rapidly diffusing particles would therefore support the lipid-flow model, whereas its absence would be evidence against it.

Fluorescence photobleaching recovery (FPR) measurements have demonstrated both rapidly and slowly diffusing (or immobile) states of concanavalin A (con A) receptors on macrophages⁷. Even the mobile particles diffused too slowly, however, to be limited by only lipid viscosity and so must be restrained by additional, possibly cytoskeletal, forces^{8,9}. FPR measurements do not discriminate between the two models because both predict that membrane glycoproteins tagged with multivalent ligands could exist in both rapidly and slowly diffusing states.

Previous observations have revealed the rearward drift of large particles on the surfaces of motile cells^{10–12} but have not determined the role of lipid flow. A recently developed method, which measures the positions of smaller particles using video-enhanced microscopy^{13,14}, provides a more discriminating test. This method, developed by Gelles *et al.*¹⁵, yields dynamic (30 Hz), precise measurements of the positions of 40-nm-diameter gold particles on a cell surface. We have observed that con A-coated gold particles bound to the surface of mouse peritoneal macrophages reversibly transfer between two modes, one of random diffusion at rates consistent with earlier FPR measurements but not undergoing rearward drift, and the other of rearward drift with slower diffusion. Both qualitative and quantitative considerations of these observations argue against the lipid-flow mechanism and in favour of a cytoskeletal model.

Glycoprotein diffusion and transport

When we added con A-coated gold particles to macrophages, they bound in a con A-dependent manner with two distinct modes of motion, random diffusion and directed transport

toward the nucleus. Although we observed both modes over the whole cell surface, we analysed particle movement on lamellipodial regions because of their more favourable optical properties (Fig. 1). As with previous observations of capping and endocytosis, particles which systematically moved rearward to sites near the endoplasm region were then internalized. After endocytosis, the particles often moved rapidly along linear paths within the cell as if transported on microtubules^{16,17}. Although most of the smaller particles diffused randomly immediately after binding, the larger aggregates of gold particles were generally transported rearward.

The qualitative difference between these two states is immediately apparent in the plots of the x - y positions of the same particle undergoing either random diffusion (Fig. 2a) or directed transport (Fig. 2b). Transitions between these two states were readily apparent and rapid (see Fig. 2c for an example of transitions). Eventually all particles experience periods of directed transport. These qualitative observations are more consistent with a cytoskeletal model because the reversible change in mode could simply correspond to reversible formation or disruption of interactions between the membrane particles and the cyto-

skeleton. Quantitative analysis of the particle trajectories, yields further information about the mechanism of motion.

Analysis of glycoprotein movement

Detailed analysis of the x and y particle positions with time yields information about diffusive and directed motions (either membrane flow or transport by cytoskeleton) of con A receptors. Computing the mean-square displacement, $\rho(\tau)$, of the particle from its initial position for different time intervals τ , yields a simple and informative representation of the data: $\rho(\tau) = \langle (r - r_0)^2 \rangle$ where r and r_0 are the positions of the particle in measurements separated by time interval τ and $\langle \dots \rangle$ indicates an ensemble average. For a particle which is both diffusing with diffusion coefficient D and undergoing directed movement at velocity V

$$\rho(\tau) = 4D\tau + (V\tau)^2 \quad (1)$$

where $4D\tau$ and $(V\tau)^2$ are the contributions from diffusion and directed movement, respectively. Hence a plot of $\rho(\tau)$ versus τ yields a straight line with slope $4D$ for a diffusing particle and an upward curving parabola (with second derivative $d^2\rho(\tau)/d\tau^2 = 2V^2$) for a particle undergoing directed movement as well as random diffusion (Fig. 4). If particle diffusion is

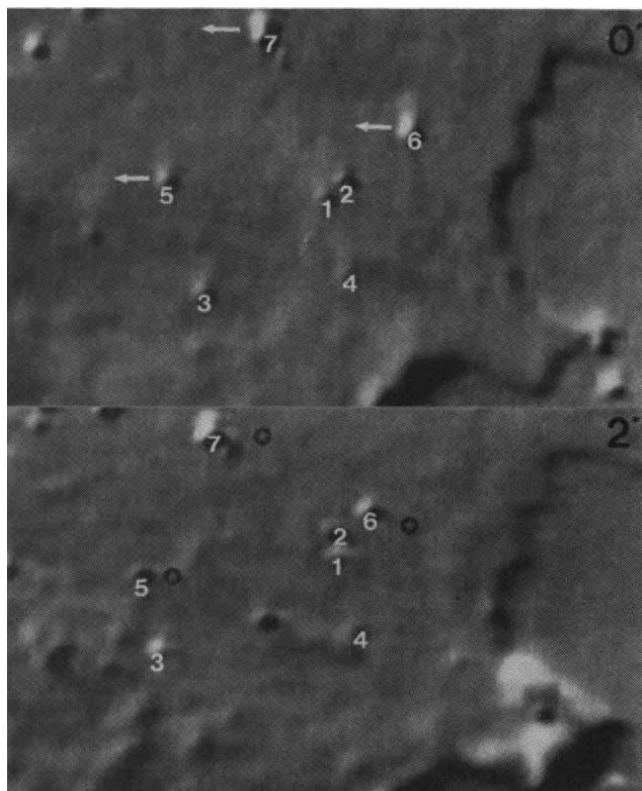


FIG. 1 Video micrographs of the lamellipodial region of a mouse bone-marrow macrophage showing the two modes of movement of gold particles bound to the surface. Particles 1, 2, 3 and 4 were diffusing randomly, whereas particles 5, 6 and 7 were undergoing directed translocation towards the rear of the cell (direction of movement noted by white arrows; original position denoted by stars). Macrophages were plated on glass coverslips in MEM (minimal Eagle's medium) with 5% fetal calf serum. After 2-3 days, coverslips were washed into HEPES-buffered MEM without phenol red indicator dye and with 2 mg ml^{-1} bovine serum albumin. Con A-gold was prepared by incubating 40-nm gold particles (Janssen Pharmaceuticals) with 2 mg ml^{-1} of concanavalin A in Dulbecco's PBS (phosphate buffered saline) for 30 min at 4°C . Con A-gold was washed free of unbound con A by centrifugation ($10,000g$ for 15 min) and resuspension in PBS-bovine serum albumin (2 mg ml^{-1}) three times. Particles were resuspended in 1/10th of the original volume of the gold suspension and sonicated to dissociate aggregates. The particle suspension was diluted 1:3 with HEPES-buffered MEM on the coverslip, sealed and the coverslips observed by video-enhanced DIC microscopy²⁷.

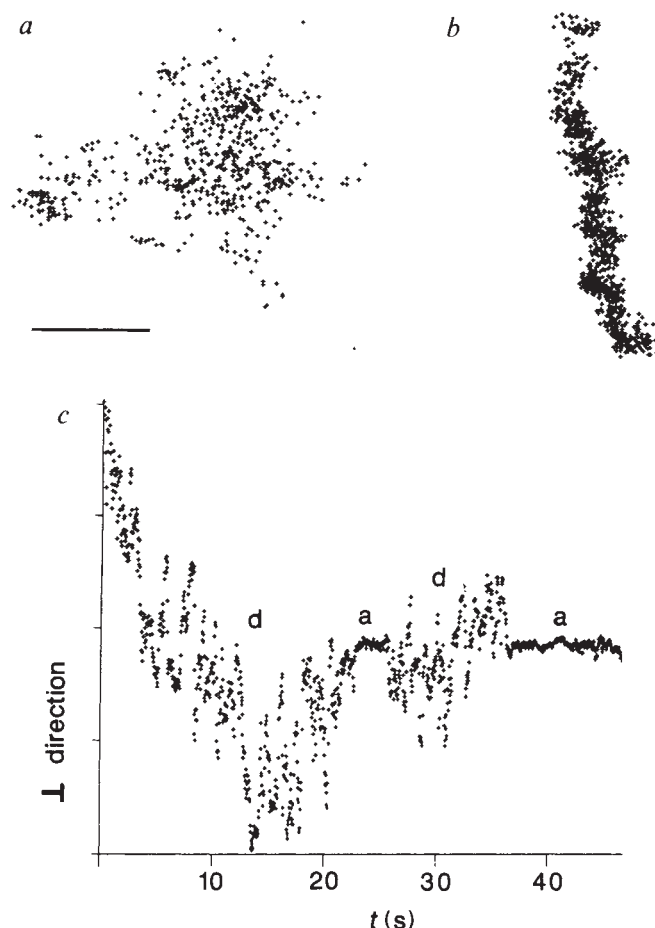


FIG. 2 The positions (x and y) of a gold particle which was initially diffusing (a) and subsequently underwent directed motion (b) towards the rear of the cell. Scale bar, 200 nm. Plot c shows the position of a particle perpendicular to the direction of transport versus time (same scale as a and b). Sequences of diffusion and directed transport are denoted by d and a, respectively. The positions were determined for every video frame (30 Hz) by the method of Gelles *et al.*¹⁵. A stationary bead bound to the glass was used as a reference point to control for stage drift. Images were first recorded on 3/4-inch video tape and then transferred to optical memory disk before position determination. A total of 750 and 1,200 points were plotted in a and b, respectively.

TABLE 1 Diffusion behaviour of con A-gold

Particle type	Parallel		Perpendicular		Two-dimensional	
	$V^2 \times 10^{+12}$ (cm s ⁻¹) ²	D (cm ² s ⁻¹)	$V^2 \times 10^{+12}$ (cm s ⁻¹) ²	D (cm ² s ⁻¹)	$V^2 \times 10^{+12}$ (cm s ⁻¹) ²	D (cm ² s ⁻¹)
Diffusing	-4.1 ± 3.0	4.0 ± 2.1 × 10 ⁻¹¹	+0.8 ± 3.3	3.2 ± 1.4 × 10 ⁻¹¹	0	3.6 × 10 ⁻¹¹
Directed	+2.3 ± 0.5	1.8 ± 0.6 × 10 ⁻¹²	-0.5 ± 0.9	1.4 ± 1.1 × 10 ⁻¹²	1.3	1.6 × 10 ⁻¹²
Stationary	-1.3	4.1 × 10 ⁻¹⁴	-0.0006	4.8 × 10 ⁻¹⁴	0	4.5 × 10 ⁻¹⁴

Particle position measurements were analysed to produce the plots of the mean-square displacement as a function of time. These were fitted to equation (1) by a quadratic curve-fitting program (see Figs 4, 5). For these analyses, the individual particles were tracked for 890–2,500 frames (total for 11 particles of 19,400 frames) and positions were referenced to stationary beads in the same frames. The values of $\rho(\tau)$ were determined for intervals ranging from $\tau=5$ –20 s, depending on the length of the data record. The value of D was determined from the initial slopes of the plots of $\rho(\tau)$ versus τ . Although flow and long-range constraints on diffusion can respectively cause positive and negative curvature (positive and negative values of V^2), they do not affect the initial slope of the $\rho(\tau)$ versus τ plots. Hence the diffusion coefficient is well determined from the initial slope of the quadratic plots. The calculated values of V^2 and D from 11 diffusing particles and 5 particles undergoing directed movement were averaged. The stationary-bead coordinates were referenced to a second stationary bead in the same frame.

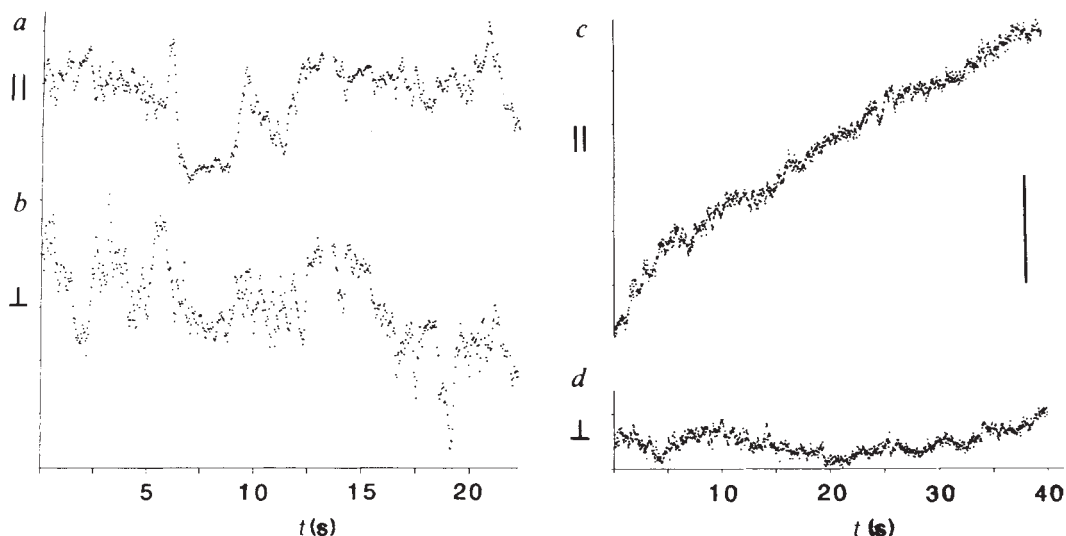
restricted, for example by barriers which stop motion beyond some distance, the plot of $\rho(\tau)$ versus τ curves downward (see Fig. 4). Then the negative V^2 term obtained by fitting equation (1) over the entire time range has no physical meaning. Nevertheless, an accurate value of D is obtained by fitting the early part of the curve, which is unaffected by the restrictions which cause the negative curvature at later times. The accuracy with which D and V are determined from equation (1) increases with the length of the data record. Even if the individual particle positions are measured with unlimited accuracy, uncertainty in D arises from the stochastic nature of diffusion. The values of $\rho(\tau)$ are less accurate for larger τ . We calculate that the stochastic uncertainty in $\rho(\tau)$ for larger τ values in our individual measurements of D is ~20%, yielding a conservative estimate of overall error of 5–8% (Table 1).

To estimate the contribution of flow to $\rho(\tau)$, we compared plots of $\rho(\tau)$ versus τ for simple diffusion (with the measured D in Table 1) and for diffusion with flow at the flow velocity of the particle in Fig. 3b. As shown in Fig. 4, measurement of $\rho(\tau)$ with a precision of 20% provides a statistically significant measurement of flow at the expected velocity. Data records from several particles with an accumulated duration of more than 600 s were therefore used (see Table 1 caption). Errors in position measurement could present another problem for the analysis. A plot of $\rho(\tau)$ versus τ for a stationary bead, however, shows no significant deviation from the baseline (Fig. 4 and Table 1). This method of analysis will, therefore, detect a directed movement of the receptors and measure the velocity.

For the diffusing particles (chosen by the criterion in Fig. 2c) the plots of $\rho(\tau)$ (in two-dimensions) versus τ were linear (Fig. 5), indicating that these particles were indeed diffusing randomly. The diffusion coefficients calculated from the slopes of such plots for all particles analysed were in the range $(1-10) \times 10^{-11}$ cm² s⁻¹ (Table 1) in rough agreement with, but generally lower than, the values obtained by FPR measurements⁷. These rates are 10–500 times slower than for the same glycoproteins in membrane blebs, in pure lipid bilayers^{8,18} or for con A-gold particles on fish keratocytes (see accompanying article by Kucik *et al.*¹⁹). Also, gold particles free in solution diffuse nearly a thousand-fold faster than the fastest movements when bound to membrane glycoproteins. It is, therefore, unlikely that the viscous resistance of the surrounding aqueous medium significantly influences the diffusion rate. The substantial increase in diffusion rate of proteins in membrane blebs⁸ and erythrocytes with an altered cytoskeleton⁹ suggests a role for cytoskeletal interactions. Recent measurements on genetically engineered proteins, however, suggest that the putative interactions are indirect^{20–22}. Although the analysis of particle movements yields slightly lower diffusion coefficients for con A receptors than does FPR, this relatively slow random motion should facilitate detection of directed transport in a membrane flow because particles would diffuse less against the flow.

Directly transported particles moved towards the nucleus in the direction expected for membrane flow. Diffusing particles on the same lamellipodia as transported particles were analysed for movement parallel and perpendicular to the direction of

FIG. 3 Plots of the particle positions versus time for axes parallel (||) (a, c) and perpendicular (⊥) (b, d) to the direction of active movement (determined by a linear least-squares fit of Fig. 2b). Left graph, diffusing particle; right graph, transported particle. Scale bar, 200 nm.



directed transport. We measured the end-to-end displacements of diffusing particles over 89 non-overlapping 20-second intervals and found the mean parallel and perpendicular displacements were insignificantly different from 0 (parallel, 10 ± 49 nm and perpendicular, 47 ± 55 nm (s.e.m.)). These observations indicate that even a lipid flow of 5 nm s^{-1} in the parallel direction is improbable ($P < 0.05$), and show that there is no bulk movement of membrane at the rate of the rearward transport. In addition, the negative curvature of the parallel plots of $\rho(\tau)$ versus τ showed that diffusion was restricted along that axis (Fig. 5a; Table 1). The absence of directed motion in diffusing particles chosen adjacent to others which were transported rearward is inconsistent with the lipid-flow model. Hence, diffusing particles are not floating freely in a lipid bilayer which is flowing at the velocity of those particles undergoing directed transport.

Glycoproteins undergoing directed transport

Analysis of the position measurements in Figs 2b and 3d shows that the path of a particle undergoing directed transport is very linear with little off-axis movement. Fitting to equation (1) provided both the drift velocities and the diffusion rates of these particles. Drift velocities agree with estimates from direct measurement of the slopes of the position versus time plots (for example, Fig. 3c). The apparent diffusion coefficients are 20-fold less than for the randomly diffusing particles (Table 1), consistent with the low amplitude of off-axis movements. Because apparent diffusion coefficients for parallel movements are the same as for perpendicular, the random component of directed transport appears isotropic. The particles rapidly and reversibly decrease their diffusion rate by 95% when they begin active movement towards the rear of the cell (Fig. 2c); therefore, it is likely that they are binding to a much larger structure which is moving rearward, like the cytoskeleton^{3,4,5}.

Effects of cytoskeletal inhibitors

If rearward transport is due to attachment to the cytoskeleton then disruption of actin or microtubule organization might block

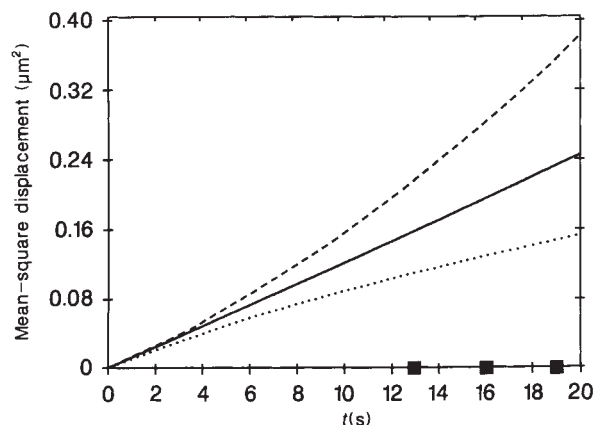


FIG. 4 Theoretical plots of the mean square-displacement versus time are presented for pure diffusion (straight lines), diffusion superimposed with flow (dashed lines) and diffusion in a cage (dots). The diffusion coefficient used in these calculations was that observed for con A-gold ($D = 3.0 \times 10^{-11} \text{ cm}^2 \text{ sec}^{-1}$) and the velocity of membrane flow was taken from the slope in Fig. 3c ($V = 20 \text{ nm s}^{-1}$). The caged particle was restrained in a $1\text{-}\mu\text{m}$ square cage. Because of errors in the measurement of the particle positions, a stationary reference particle would also show a diffusion-like motion but with a much smaller diffusion coefficient, $D = 4.45 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$ (black squares). The expected relative error in each data point in the pure diffusion curve is $\leq 20\%$ for most measurements. Therefore, the relative error in D computed by fitting a sequence of these points should be much lower. Nevertheless, the standard deviation in the experimentally measured diffusion coefficients is $\sim 50\%$ (Table 1), indicating that members of the diverse population of con A receptors diffuse at various rates.

this movement. This was tested using the cytoskeletal inhibitors nocodazole and cytochalasin D, which disrupt microtubules and microfilaments, respectively. Rearward movement of particles was not altered by nocodazole at concentrations which blocked all directed intracellular organelle movements. By contrast, cytochalasin D blocked rearward movement of surface-bound particles while internal organelle movements persisted. Integrity of the actin-filament system but not of microtubules, therefore, is required for retrograde transport of surface-bound particles. This conclusion is also consistent with the inhibition by cytochalasins of retrograde movements of particles on fibroblasts⁵ and neuron growth cones^{4,23}. It is likely that the rearward transport mechanism is the same in all these systems^{3,23,24}.

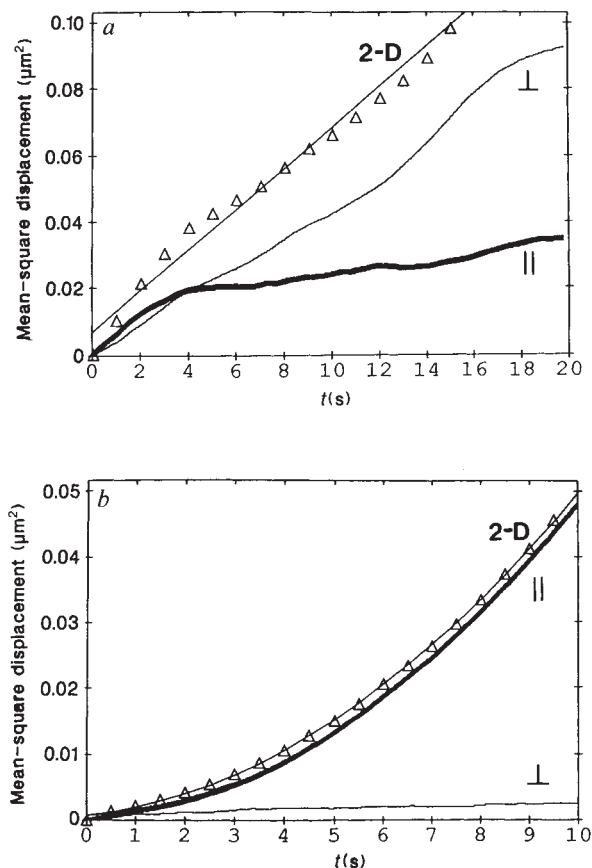


FIG. 5 The measured mean-square displacements of a randomly diffusing particle (a) and a particle undergoing directed transport (b). Using the parallel axis as that of directed transport, the displacements are plotted for the parallel (||) and perpendicular (⊥) axes as well as for two-dimensional displacement (Δ). a, Negative curvature of parallel displacement versus time indicates that diffusion is restricted, in sharp contrast to the positive curvature in b. The initial linear portion of plot a (two-dimensional component) can be represented as $\rho(\tau) = 6.120\tau + 6.890$, yielding $D = 1.5 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$. In b, there is directed motion along the parallel axis but not in the perpendicular direction. Fitting to equation (1), $\rho(\tau) = 405\tau^2 + 850\tau + 714$ yields $D = 2.1 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$ and $V = 20 \text{ nm s}^{-1}$. In a 20-s period the end-to-end distance for a particle with $D = 1.5 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$ should be 346 nm and, if there were a global flow with $V = 20 \text{ nm s}^{-1}$, the same particle should also have drifted ~ 400 nm. This was not observed. The observation of a plateau in $\rho(\tau)$ can be interpreted in terms of the restriction of diffusion to a limited domain (of dimension ~ 350 nm; H.Q. *et al.*, manuscript in preparation). This domain itself could be systematically transported. Within the statistical accuracy of the measurements, however, the velocity of transport must be much less than 4 nm s^{-1} . The significant non-zero offset on the y-axis in a, when $t=0$, can be explained by high-frequency noise or local fast diffusion, which is of the order of $5 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$.

In principle, the longer the correlation analysis is extended, the easier it is to discriminate the contributions of flow to the trajectories of the diffusing particles. It is also important, however, to take account of the decreasing accuracy of the longer time points because of the limited number of data points. It turns out that for a data set with, say, N time points at intervals Δt , the relative error (r.m.s. deviation/mean) of $\rho(\tau)$ at $\tau = n\Delta t$ varies as $0.82(N/n)^{-1/2}$. Hence we have chosen to extend $\rho(\tau)$ out to τ values which give a relative error of less than $\sim 20\%$.

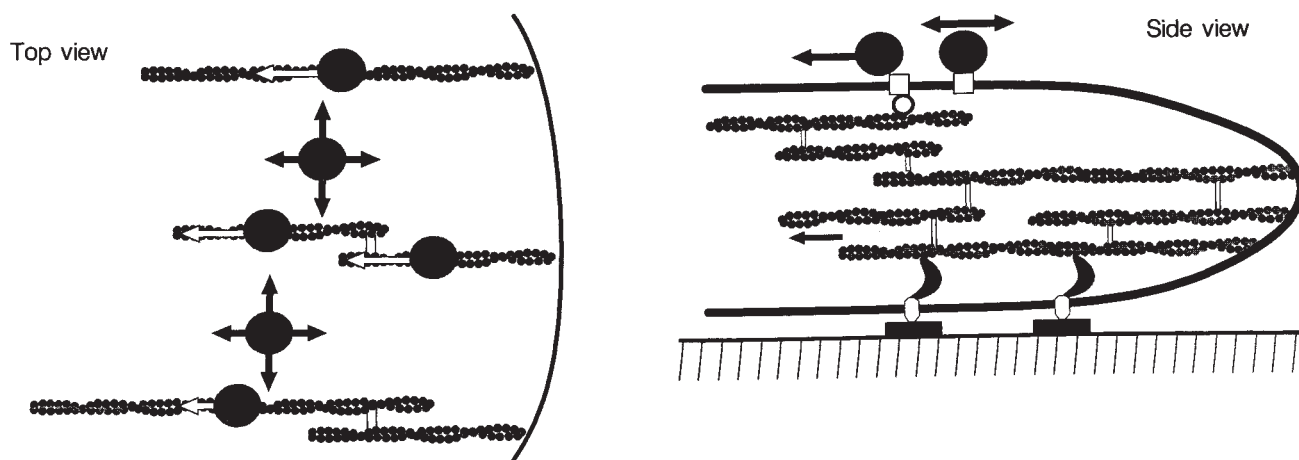


FIG. 6 Model for directed transport of particles without the induction of a membrane flow. The actin cytoskeleton is moving rearward (after earlier models)^{24,25}. Gold particles on the dorsal surface are either attached indirectly to the cytoskeleton and moving rearward, or free and diffusing

randomly. Note that if no membrane is added at the front of the lamellipodium, then the bulk of the membrane over the lamellipodium is stationary if the leading edge is stationary, or flowing forwards if the edge is moving forwards.

Discussion

The rapid and precise determination of glycoprotein position allows the diffusion and flow of membrane proteins to be measured directly. Although the gold-tagged receptors diffuse at a slightly slower rate than fluorescently tagged receptors in FPR studies, the slower diffusion rates allow more sensitive detection of membrane flow. The normal video framing rate of 30 Hz misses rapid motions, but the method is sensitive to diffusion rates of 10^{-10} – 10^{-13} cm² s⁻¹, the observed range (see Fig. 4).

Previous studies of plasma membrane glycoprotein diffusion by FPR and other methods have shown the presence of diffusing and 'immobile' fractions which could correspond to the two modes of movement observed in these studies. Those methods, however, could not determine if the same molecules changed reversibly from a diffusing to an immobile state. Transitions of gold-tagged receptors between modes of random movement and directed transport commonly occurred rapidly, suggesting that glycoproteins are continually binding and releasing from cytoskeletal sites. Further studies are under way to analyse the processes which control the transitions between the two modes.

It is striking that, among neighbouring tagged receptors, some diffuse randomly while others are systematically transported rearward. In fact, the negative V^2 values observed for randomly diffusing particles along the axis of directed rearward transport suggest that diffusion in this direction is bounded; movements in the perpendicular direction seem to be simply diffusive. These observations raise the question of whether membrane is being

added to the leading edge of lamellipodia. In the lamellipodial regions there are very few membrane vesicles which could fuse with the leading edge to drive a membrane flow. The observations of viral glycoprotein entry into the front of the endoplasm and to the front of the lamellipodium^{2,3}. Another possibility is that proteins are actively transported forwards, as observed in fish keratocytes¹⁹.

Actin filaments in the lamellipodial region of the cell are known to be moving rearward. The cytochalasin D block of the rearward movement of tagged receptors in this region is, therefore, consistent with the view that the receptors are reversibly attaching to the actin cytoskeleton (Fig. 6). Cortical contraction³ would draw actin to the membrane towards the rear of the cell. Membrane glycoproteins could then be transported by direct or indirect attachment to actin. Ishihara *et al.*²⁵ have observed a steady-state gradient in concentration of a fluorescently labelled membrane glycoprotein, which could be interpreted either in terms of a 'retraction-induced spreading' model or a membrane-flow model²⁶. In this context, reversible attachment of glycoproteins to a contracting cortex could produce the gradient. Using a popular analogy of biological membranes as oceans with protein logs floating in them, these results indicate that directed movement of a subset of logs does not need to produce a current in the ocean which would carry other logs along (Fig. 6). Certainly the fluidity of the lipid phase is sufficient to allow independent movement of membrane glycoproteins. □

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1. Bretscher, M. S. *Science* **224**, 681–686 (1984).
2. Singer, S. J. & Kupfer, A. A. *Rev. Cell Biol.* **2**, 337–365 (1986).
3. Bray, D. & White, J. G. *Science* **239**, 883–888 (1988).
4. Forscher, P. & Smith, S. J. *J. Cell Biol.* **107**, 1505–1516 (1988).
5. Fisher, G. W., Conrad, P. A., DeBiasio, R. L. & Taylor, D. L. *Cell Motil. Cytos.* **11**, 235–247 (1988).
6. Wang, J.-L. *J. Cell Biol.* **101**, 597–602 (1986).
7. Henis, Y. I. & Elson, E. L. *Expl. Cell Res.* **136**, 189–201 (1981).
8. Jacobson, K., Ishihara, A. & Inman, R. A. *Rev. Physiol.* **49**, 163–175 (1987).
9. Sheetz, M. P. *Sem. Hematol.* **20**, 175–188 (1983).
10. Abercrombie, M., Heaysman, S. E. M. & Pegrum, S. M. *Expl. Cell Res.* **62**, 389–398 (1970).
11. Harris, A. K. in *Locomotion of Tissue Cells*, Ciba Fdn Symp. **14**(d) 3–20 (1973).
12. Dembo, M. & Harris, A. K. *J. Cell Biol.* **91**, 528–536 (1981).
13. Allen, R. D., Allen, N. S. & Travis, J. L. *Cell Motil.* **1**, 291–302 (1981).
14. Inoué, S. *J. Cell Biol.* **89**, 346–356 (1981).
15. Gelles, J., Schnapp, B. J. & Sheetz, M. P. *Nature* **331**, 450–453 (1988).

16. Schroer, T. A. & Sheetz, M. P. *Cell*, in the press.
17. Vale, R. D. A. *Rev. Cell Biol.* **3**, 347–378 (1987).
18. Saffman, P. G. & Delbruck, M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3111–3115 (1975).
19. Kucik, D. F., Elson, E. L. & Sheetz, M. P. *Nature* **340**, 315–317 (1989).
20. Edidin, M. & Zuniga, M. *J. Cell Biol.* **99**, 2333–2335 (1984).
21. Livneh, E. *J. Cell Biol.* **103**, 327–331 (1986).
22. Scullion, B. F., Hou, Y., Puddington, L., Rose, J. K. & Jacobson, K. J. *J. Cell Biol.* **105**, 69–75 (1987).
23. Mitchison, T. & Kirschner, M. *Neuron* **1**, 761–772 (1988).
24. Smith, S. J. *Science* **242**, 708–715 (1988).
25. Ishihara, A., Holifield, B. & Jacobson, K. J. *J. Cell Biol.* **106**, 329–343 (1988).
26. Bretscher, M. S. *J. Cell Biol.* **106**, 235–237 (1988).
27. Schnapp, B. J. *Meth. Enzym.* **134**, 561–573 (1986).

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Large scale extended X-ray emission from the Virgo cluster of galaxies

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HOT, tenuous gas is revealed, by X-ray observations¹, to permeate many clusters of galaxies. If the gas is in hydrostatic equilibrium, it traces the cluster's gravitational potential. X-ray mapping of clusters of galaxies can be used to estimate the total cluster mass, including dark matter, as well as its spatial distribution. Previous observations of X-ray emission from the Virgo cluster, the nearest rich cluster of galaxies, showed it to be concentrated within ~ 100 arcmin of M87, the dominant galaxy in the cluster^{2,3}. Because the optical image of the Virgo cluster is $\sim 12^\circ$ in diameter, the X-ray emission was thought to be associated with M87 rather than with the cluster as a whole. Here we report a preliminary mapping of Virgo made by the Ginga satellite which shows the extended X-ray emission as broadly distributed as the cluster's optical extent. This indicates that the galaxies of the Virgo cluster are immersed in a hot diffuse intracluster medium.

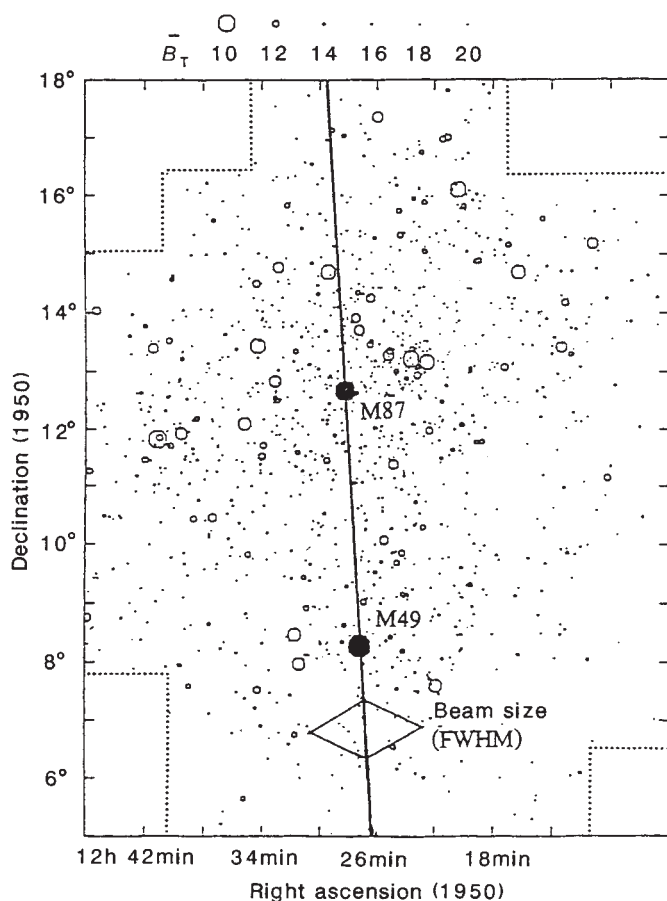


FIG. 1 The scan path of the Ginga LAC superimposed on the map of the member galaxies of the Virgo cluster⁷. The diamond indicates the FWHM field of view of the LAC experiment. The galaxies form two subclusterings around M87 and M49. The symbol size shows the apparent blue magnitude (B_T) of the galaxy.

The Virgo cluster of galaxies is generally classified as an irregular cluster⁴, in which the distribution of galaxies is not yet relaxed. As the galaxies are away from dynamical equilibrium, we must rely on X-ray observations to map the distribution of the gravitating mass, but the large angular extent of the Virgo cluster has made it difficult to carry out an X-ray study of the whole cluster. Previous observations with Einstein IPC and Exosat ME^{2,3} were restricted within 100 arcmin of the dominant galaxy M87, and the mass estimate was only available in the inner portion of the cluster around M87. Because the galaxies are not yet relaxed, a correlation study of the distributions of the gravitating mass and the galaxies might give important information about galaxy formation. Thus, highly sensitive X-ray observations of the whole Virgo cluster capable of searching and mapping large-scale diffuse X-ray emission were necessary.

We have been conducting mapping observations of the Virgo cluster of galaxies with the Large-Area-Counters (LAC) experiment⁵ on board the X-ray-astronomy satellite Ginga. The LAC experiment consists of eight identical proportional counters that are sensitive in the 1.5–38 keV energy range and have a total effective area of 4,000 cm² (ref. 6). The field of view of the LAC is defined by the mechanical collimator to be $1^\circ \times 2^\circ$ (FWHM, full width at half maximum). Because of its high throughput (detector area $\times$ solid angle), the LAC is quite sensitive to diffuse X-ray emission down to a limit of $\sim 10^{-9}$ erg s⁻¹ cm⁻² sr⁻¹, which is nearly ten times better than any previous instrument.

The initial mapping observation of the Virgo cluster was made during 6–9 June 1988, when the path through M87 and M49 was scanned in one dimension. In Fig. 1 we show the scan path of the LAC as well as its beam size (FWHM) superimposed on the map of member galaxies. A recent optical study⁷ revealed that the structure of the Virgo cluster has two subclusterings around M87 and M49, and we chose the scan path so that it ran through them.

Figure 2 shows the LAC count-rate histogram in the energy range 1.5–4.5 keV along the scan path, after subtraction of non-X-ray background events and the cosmic X-ray background. The X-ray emission is strongly peaked at M87 as noted in previous works^{2,3}. The solid line in Fig. 2 indicates the point-source collimator transmission profile of the LAC experiment, normalized to the peak intensity. It is significant that the X-ray emission is extended compared to the beam width. The data around M87 were compared to and found to be consistent with the surface brightness distribution observed from the Einstein IPC². The hump at 4.4° south of M87 corresponds to the elliptical galaxy M49. The most remarkable result of our observation is the detection of large-scale extended X-ray emission up to about 7° in the south from M87 and up to about 5° in the north.

Using several high-latitude background pointings of $\sim 10^4$ s exposure, we checked the stability of the LAC non-X-ray background during scanning observations. Assuming the same scanning speed as that employed in the Virgo cluster mapping, false

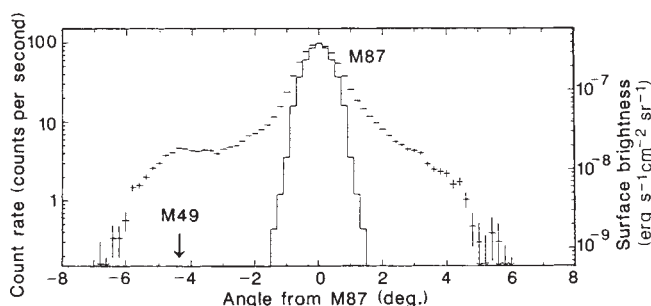


FIG. 2 The Ginga LAC count-rate histogram (1.5–4.5 keV X-rays) along the path shown in Fig. 1. The solid line is the point-source collimator transmission profile of the LAC experiment, normalized to the peak intensity. The position angle of M49 is indicated by an arrow.

attitude data were given to these pointings. Each pointing was processed using the same procedure as that used for mapping the Virgo cluster and the resultant count-rate histogram, which should be constant, was examined based on the hypothesis of constant count rate. This hypothesis was not rejected by χ^2 test at 90% confidence level. The non-Poisson r.m.s. fluctuation of the count rate in the 1.5–4.5 keV range was calculated for each count-rate histogram and always found to be less than 0.1 counts per second. We conclude that the large-scale extended X-ray emission shown in Fig. 2 is not an artefact due to background variation or incorrect background subtraction.

Fabricant and Gorenstein² analysed a mosaic of the Einstein IPC images of the Virgo cluster. Using the Exosat ME detector, collimated proportional counters of 45 arcmin (FWHM) square field of view, the X-ray emission from the Virgo cluster has been mapped³. These authors measured the X-ray surface brightness out to 100 arcmin from M87. At larger radii, X-ray emission faded away below detection limit. Since the X-ray emission was strongly peaked at M87, these authors associated it with the massive dark halo around M87 itself, rather than with the cluster as a whole. Our Ginga observation shows that the X-ray-emitting

intracluster medium in the Virgo cluster extends farther and is as broadly distributed as the cluster's optical extent.

Although our observation is restricted to one path through the Virgo cluster, this positive detection of widely extended X-ray emission is promising. With simple assumptions of spherical symmetry and isothermality, we estimate that the total gravitating mass in the whole cluster is at least three times larger than that estimated within 100 arcmin from M87. The quantitative estimate of the mass of the X-ray-emitting intracluster medium and the total gravitating mass, as well as their spatial distribution, requires a complete two-dimensional observation of the whole cluster, and this is now under way. □

Received 18 April; accepted 12 June 1989.

1. Sarazin, C. L. *Rev. Mod. Phys.* **58**, 1–116 (1986).
2. Fabricant, D. & Gorenstein, P. *Astrophys. J.* **267**, 535–546 (1983).
3. Edge, A. C., Stewart, G. C. & Smith, A. *Proc. NATO Advanced Study Institute on Hot Thin Plasmas in Astrophysics* (ed. Pallavicini, R.) 335–340 (Kluwer, Dordrecht, 1988).
4. Abell, G. O. *Galaxies and the Universe* (eds Sandage, A., Sandage, M. & Kristan, J.) 601–645 (University of Chicago Press, 1975).
5. Makino, F. *et al. Astrophys. Lett.* **25**, 223–233 (1987).
6. Turner, M. J. L. *et al. Publ. astr. Soc. Japan* **41**, 345–372 (1989).
7. Binggeli, B., Tamman, G. A. & Sandage, A. *Astr. J.* **94**, 261–277 (1987).

Evidence of the mid-latitude impact of Antarctic ozone depletion

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THE 1987 Antarctic Airborne Ozone Expedition established that the springtime depletion of Antarctic ozone^{1,2} is due to photochemical destruction³ following a preconditioning phase involving heterogeneous reactions on polar stratospheric clouds⁴. Ozone destruction is concentrated in the cold Antarctic stratospheric vortex of winter and early spring, where these clouds occur. Nevertheless, ozone data reveal a secular decline into mid-latitudes^{5,6}. Modelling studies^{6,7} suggest a 'dilution effect' whereby ozone-poor air is transported into mid-latitudes when the vortex breaks up in late spring (the 'final warming'). The extent to which the effect penetrates into middle latitudes is clouded by statistical uncertainty in trend analyses and by the difficulty of separating photochemical and dynamical effects on seasonal timescales. Here we analyse record low ozone values found over Australia and New Zealand during December 1987⁸ following the record low Antarctic values of October 1987⁸. In fact, there was a sudden decline of ozone amounts in mid-month; this rules out photochemical effects as a cause and allows us to investigate the underlying processes on a 'case study' basis. Using data from ozonesondes, radiosondes, the Nimbus-7 total ozone mapping spectrometer (TOMS), and meteorological analyses from the National Meteorological Center (Washington), we argue that these low values resulted from transport of ozone-poor air from higher latitudes. It therefore seems that the chemical destruction of ozone over Antarctica in early spring is having an impact on lower latitudes.

The Antarctic stratosphere of early spring is dominated by an intense westerly circulation—the 'polar vortex', within which low total ozone values are found, surrounded by a 'collar' of high ozone in mid-latitudes. Later in the spring (usually November), the polar vortex rapidly erodes and breaks up, resulting in deceleration or even reversal of the westerlies. Streams of polar air are torn from the vortex and left in the mid-latitudes as remnants of the breakup⁹. During these events, the high-ozone collar intrudes onto the pole while the minimum is displaced towards mid-latitudes. Spring 1987 was highly anomalous^{10–12}: October/November ozone and temperature were at record lows, whereas the vortex breakup (early December) was the latest recorded.

Record low total ozone values over Australia during December 1987 were reported by Atkinson and Easson⁵. A reduction of 25 Dobson units (DU) was seen at Perth on 11 December and at Melbourne one day later. Figure 1 shows TOMS data over 5 stations (Melbourne, Perth, Brisbane and Hobart, Australia and Lauder, New Zealand). The Melbourne data show a 70-DU decrease between the days 6 and 15 December, with the sharpest drop between 11 and 12 December. This feature is also evident one day earlier at Hobart and Perth and one day later

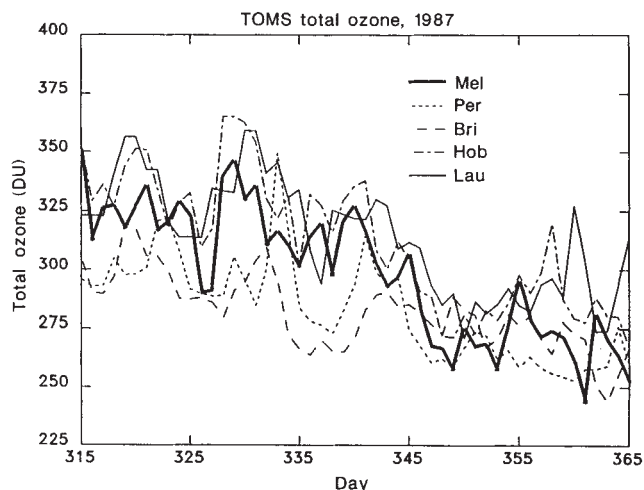


FIG. 1 TOMS total ozone values (DU) during December 1987 co-located to four Australian stations (Melbourne (38° S, 145° E), Perth (32° S, 116° E), Brisbane (27° S, 153° E), Hobart (43° S, 147° E)) and Lauder, New Zealand (45° S, 169° E).

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at Lauder. Throughout the period 11–31 December 1987, TOMS daily total ozone values at Melbourne (with the single exception of 22 December) and Perth (with the single exception of 30 December) were the lowest recorded for December at those locations since TOMS began measurement in 1979. Further north, the Brisbane data were not anomalously low (the systematic decline being normal at this time of year). On the basis of these data, we adopt 8 and 14 December as end-points for this event.

Hemispheric maps of TOMS total ozone (Fig. 2a) on 8 December show low ozone values in the polar region (less than 230 DU). These values are higher than the record low October/November values (by more than 100 DU) but much

lower than 'normal' values for late spring. An ozone maximum (part of the mid-latitude collar) was located just off the Antarctic coast (55°S , 85°E) with a minimum to its north-west at around 60°E . Just to the east of the maximum, over the next few days, a pronounced band of low ozone developed out of the polar minimum. These features moved steadily eastward, and by 14 December (Fig. 2b) the maximum was south of New Zealand with the minimum to its north-east, over and to the south-east of New Zealand. A second minimum is evident immediately to the south of eastern Australia. The spatial structure of the rapid reductions seen in Fig. 1 is shown in Fig. 2c. The large increase in the polar region is evidence of the breakup of the 'ozone hole'. Equally dramatic are the two bands of total ozone

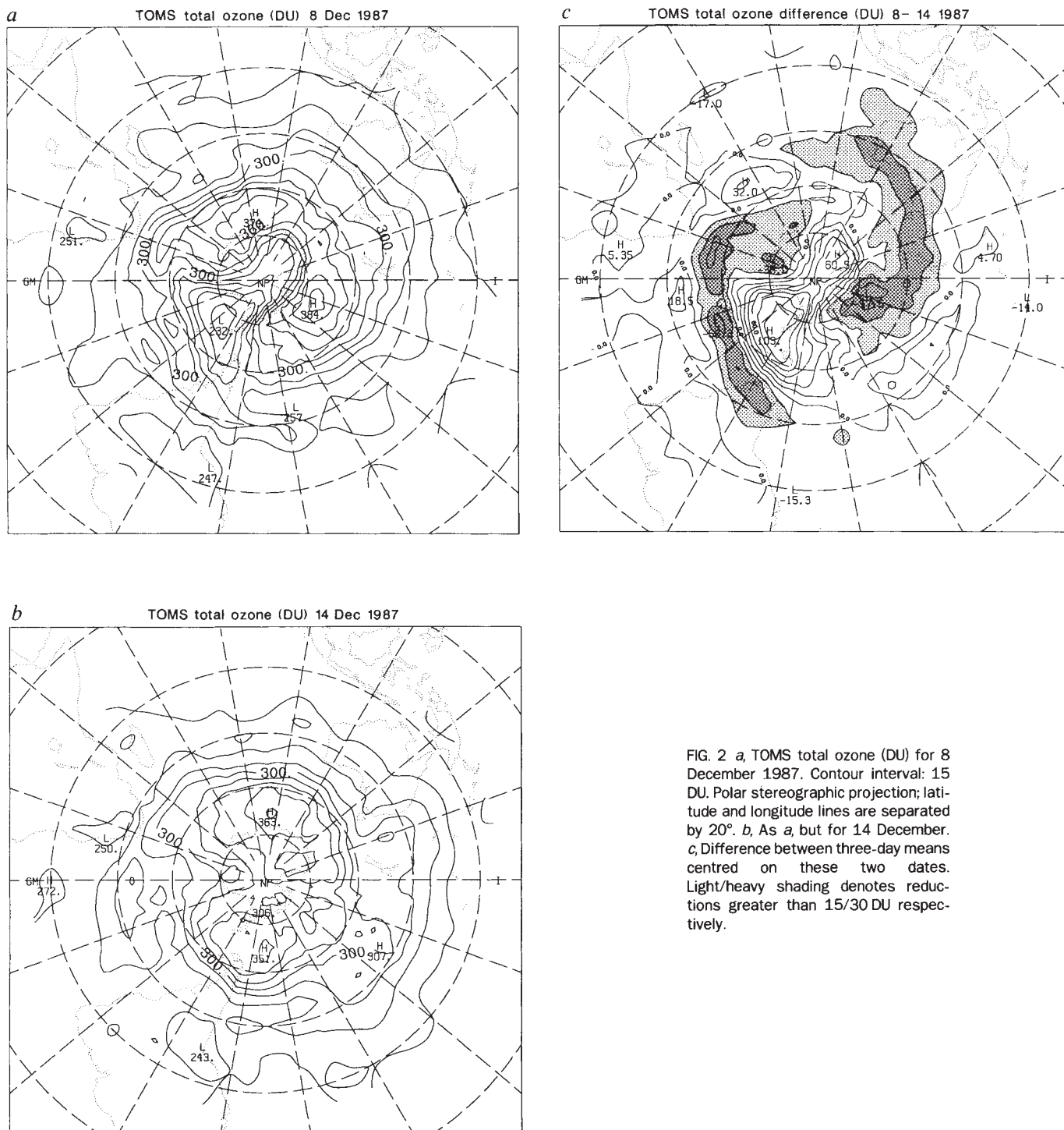


FIG. 2 a, TOMS total ozone (DU) for 8 December 1987. Contour interval: 15 DU. Polar stereographic projection; latitude and longitude lines are separated by 20° . b, As a, but for 14 December. c, Difference between three-day means centred on these two dates. Light/heavy shading denotes reductions greater than 15/30 DU respectively.

reduction, one (up to 45 DU) extending across southern New Zealand and southern Australia.

These characteristics suggest a high-latitude origin for the depleted air. We argue that the meteorological evidence supports this, although other effects probably also contributed to the observed reductions. Ozone has a long lifetime in the lower stratosphere¹³; however, total ozone (the total amount in a vertical column) is not a material tracer. It is sensitive to vertical motion through replacement of ozone-rich, high-level air by ozone-poor, low-level air. Total ozone data alone cannot discriminate between horizontal transport and vertical displacement.

The effects of vertical motion can be assessed from the ozonesonde data from Melbourne and Lauder (Fig. 3). Unfortunately no data were available from Melbourne between the 8 and 22 December (but ozone values were still low on 22 December; Fig. 1) and the Lauder sonde failed at 65 hPa on 7 December. From the partial-pressure profiles, most of the ozone reduction at Melbourne between 8 and 22 December occurred between 200 hPa (11.5 km altitude, potential temperature 360 K) and 30 hPa (24 km, 588 K). Little change is apparent at Lauder between 7 and 14 December below 65 hPa (19 km, 484 K); in fact about 75% of the observed column reduction over the period must have occurred above this level. From the profiles, an upward displacement in the lower stratosphere of about 1.5 km could have caused the observed reduction in column ozone. However, this would also cause an adiabatic cooling of about 15 K. There was indeed a lower stratospheric cooling over the region between 8 and 14 December (and its spatial structure was similar to that of the ozone changes), but of less than 5 K. As lower stratospheric heating/cooling rates are typically less than 0.5 K per day, it seems reasonable to infer that local vertical motion alone cannot account for the ozone reduction, although it may have been a contributory factor. We are therefore led to consider quasi-horizontal advection of ozone-poor air from the south.

Both Ertel potential vorticity (PV) and potential temperature are conserved under inviscid, adiabatic flow¹⁴. Maps of PV on isentropic surfaces are used to trace quasi-horizontal air movements. In practice, PV diagnostics need to be interpreted with caution, especially in the Southern Hemisphere where data coverage is sparse¹⁵. Nevertheless, our PV distributions appear meaningful on the grounds of good continuity in time and consistency with other information—in particular, with isen-

tropic trajectories determined from balanced wind fields. Here we focus on the 500-K isentropic surface, located at about 70 hPa (about 18 km) over the pole and sloping upward to about 50 hPa (about 21 km) over Australia and New Zealand. (The polar regions were warmer than mid-latitudes at this time, even though the vortex had not yet broken down.)

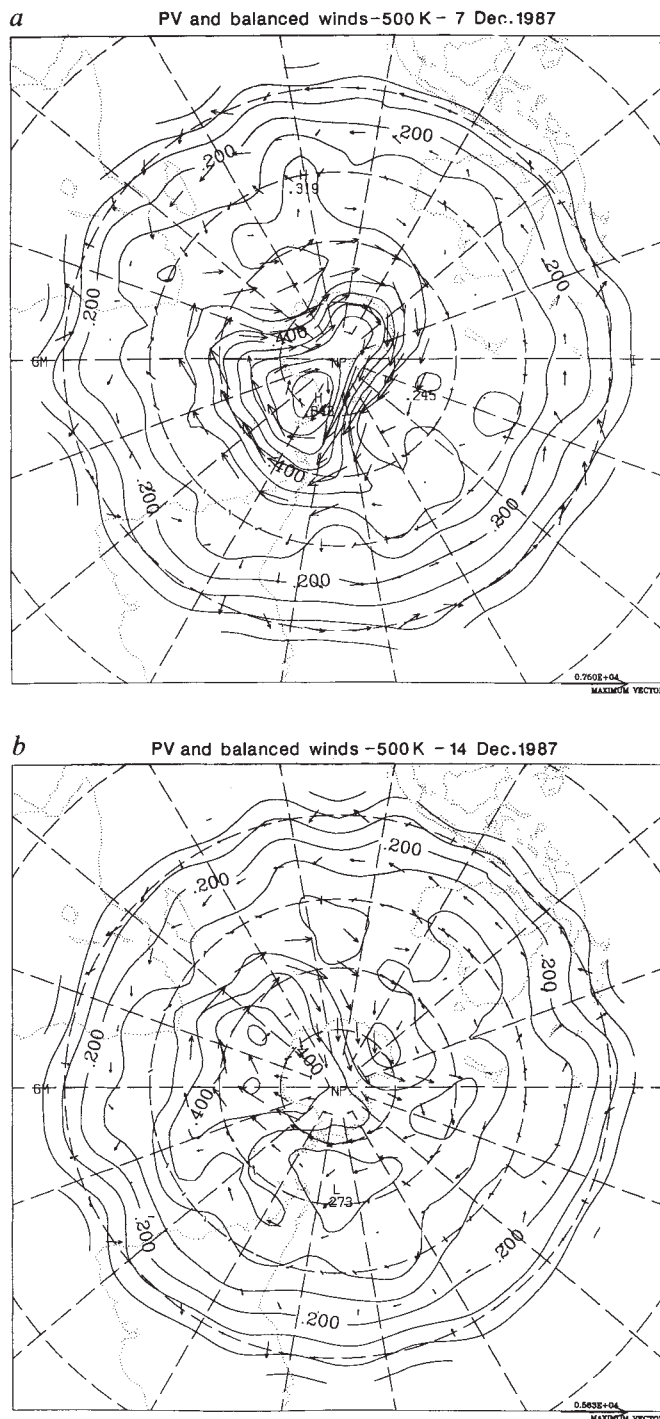


FIG. 4 Meteorological analyses of the Southern Hemisphere in early December 1987. *a*, Ertel potential vorticity (PV; contour interval $5 \times 10^{-7} \text{ K s}^{-1} \text{ Pa}^{-1}$) and balanced winds on the 500-K isentropic surface for 7 December; projection as Fig. 2. PV is defined as $-\zeta \partial \theta / \partial p$, where ζ , θ and p are respectively the vertical component of absolute vorticity, potential temperature and pressure. (The sign of PV is chosen such that cyclonic PV is positive.) *b*, As *a*, but for 14 December; note the 'tongue' of high PV across New Zealand and Southern Australia. *c*, Ten-day trajectories on the 500-K isentropic surface starting 6 December, calculated using the daily balanced

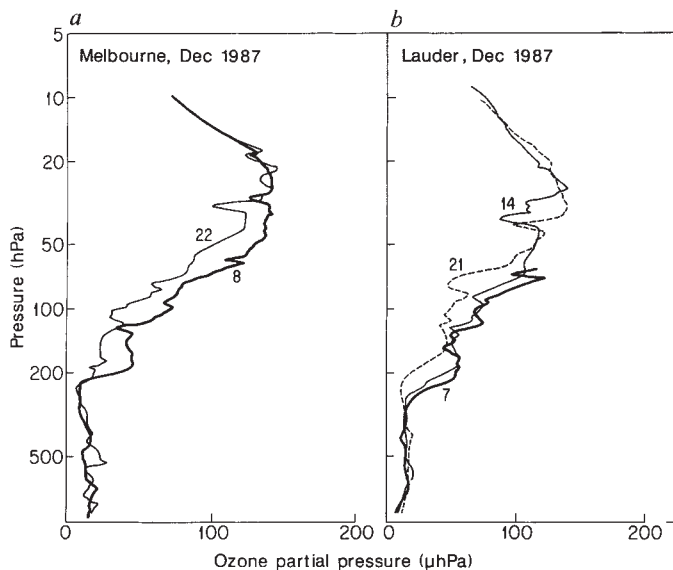


FIG. 3 Ozonesonde profiles showing ozone partial pressures for *a*, 8 and 22 December 1987 at Melbourne, and *b*, 7, 14 and 21 December 1987 at Lauder.

There was a clear association between the ozone evolution and the lower stratospheric circulation at the time of interest. Between 5 and 8 December, an anticyclone developed over the south-west Indian Ocean (the anticlockwise wind gyre near 65° S, 55° E in Fig. 4a); the associated northward flow would transport air out of the outer edges of the vortex into middle

latitudes. This is evident in the PV distribution; a tongue of vortex air extended into mid-latitudes coincident with the ozone trough seen in Fig. 2a. It seems likely that these low ozone values arose from transport of ozone-poor air out of the vortex; isentropic surfaces were relatively flat and steady over the Indian Ocean at this time, indicating the virtual absence of vertical motion. Subsequently, the anticyclone developed further and migrated south-east. This had two consequences; the westerlies redeveloped over the Indian Ocean well to the north of the anticyclone and a strong north-eastward flow developed to its south and east. By 14 December, (Fig. 4b) the corresponding PV minimum was located at about 70° S, 140° E. At this stage the polar vortex was breaking up to the south-east of New Zealand with a region of high-PV air extending as far as southern Australia.

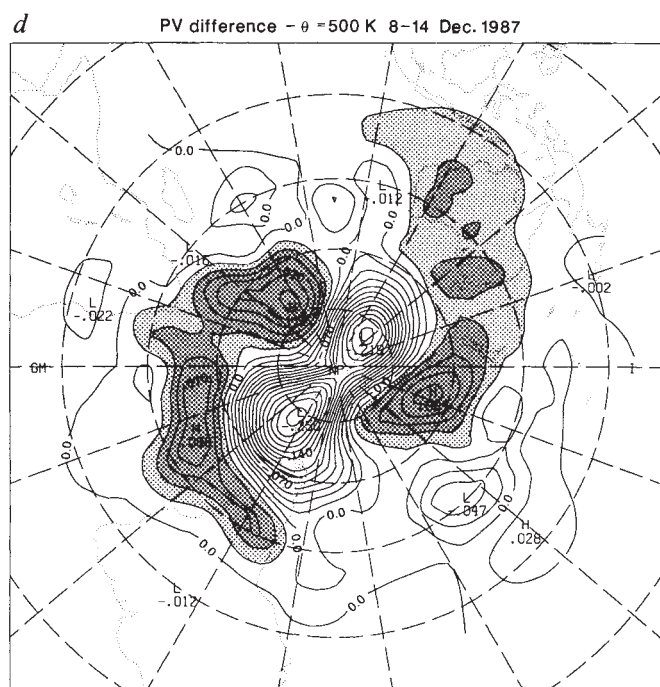
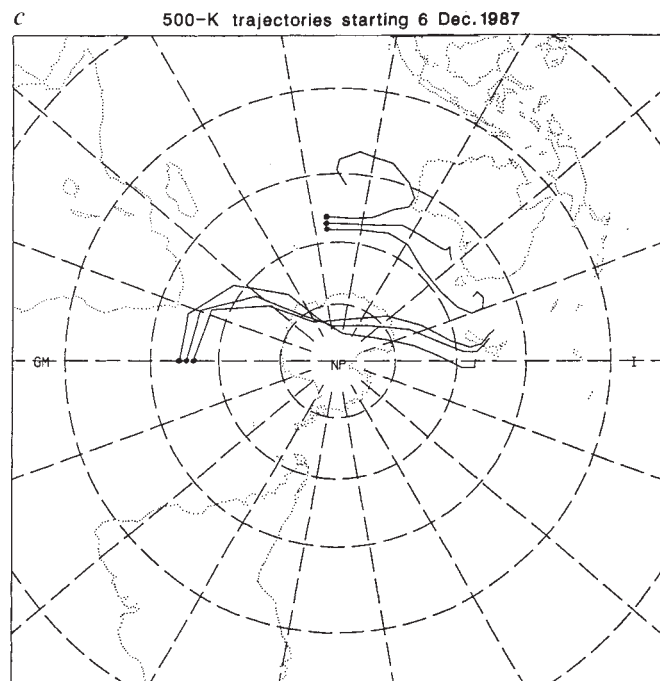
PV changes over the period 8–14 December are shown in Fig. 4d. The similarity of this figure to the corresponding total ozone change (Fig. 2c) is striking. The polar decrease and the band of increased PV extending across southern Australia and New Zealand suggest that the observed reduction of ozone in the latter region resulted from the import of high-latitude air. We note from the ozone and PV evolution and from the ground-based ozone data that the northern limit of penetration was about 30° S.

These conclusions are supported by isentropic trajectories; a selection starting from 6 December is shown in Fig. 4c. These confirm that horizontal transport occurred in the way the PV and wind analyses suggest. Trajectories over New Zealand on 14 December originate mostly from the south-west, having been advected directly from the vortex edge around the south and east of the developing anticyclone. Transport over southern Australia occurred from the west, its origin being the eastern Indian Ocean (that is, the region of reduced ozone which was identified above as the remnant of the event that began on 5 December). There were thus two routes for horizontal transport into the Australia/New Zealand region during this period.

Our conclusion is that transport of ozone-poor air from higher latitudes was the chief factor in producing the anomalously low total ozone observed over southern Australia and New Zealand. Analyses at other lower stratospheric levels were similar, although latitudinal transport was weaker below 500 K. In fact, PV advection near and above 500 K was accompanied by upwelling beneath, where it seems this upward motion made a significant contribution to local ozone reduction, although this was not a dominant component of the column change. Unfortunately, the techniques available to trace air motion (PV analyses and trajectories) are not reliable over long periods. We cannot confirm unequivocally that the ozone-poor air that moved into middle latitudes had been influenced by chemical depletion. What we have established is that air did migrate out from the vortex edge during early and mid-December. Anomalously low ozone concentrations were observed near the vortex edge in October³, so transport of this air could be enough to influence middle latitudes. There is therefore a strong *prima facie* case for believing that the anomalously low ozone values reported here were manifestations of the 'dilution effect'. □

Received 17 April; accepted 12 June 1989.

- Farman, J. C., Gardiner, B. G. & Shanklin, J. D. *Nature* **35**, 207–210 (1985).
- Stolarski, R. S. *et al.* *Nature* **322**, 808–811 (1986).
- Anderson, J. *et al.* *J. geophys. Res.* (1989).
- Toon, O. B., Hamill, P., Turco, R. P. & Pinto, J. *Geophys. Res. Lett.* **13**, 1284–1287 (1986).
- Atkinson, R. & Easson, J. in *Proc. Inter. Ozone Symp.* (eds Bojkov, R. & Fabian, P.) (A. Deepak, Hampton, Virginia, 1988).
- Sze, N. D. *et al.* *J. geophys. Res.* (1989).
- Prather, M. J. & Garcia, M. M. in *Proc. Polar Ozone Workshop, Snowmass 1988* (NASA Conf. Publ. 10014, 1988).
- Krueger, A. J., Schoeberl, M. R., Stolarski, R. S. & Sechrist, F. S. *Geophys. Res. Lett.* **15**, 1365–1368 (1988).
- Newman, P. A. *Geophys. Res. Lett.* **13**, 1228–1231 (1986).
- Randel, W. J. *Geophys. Res. Lett.* **15**, 911–914 (1988).
- Gardiner, B. J. *Geophys. Res. Lett.* **15**, 901–904 (1988).
- Newman, P. A. & Schoeberl, M. R. in *Proc. Inter. Ozone Symp.* (eds Bojkov, R. D. & Fabian, P.) (A. Deepak, Hampton, Virginia, 1988).



winds. Three trajectories were started at 85° E, on the edge of the 'breakout' air evident in a; these move eastward toward western and southern Australia. The three initialized on the Greenwich meridian break out from the vortex around 11 December and move over New Zealand. d, The 500-K EPV change between three-day means centred on 8 and 14 December (contour interval: $7.5 \times 10^{-8} \text{ K s}^{-1} \text{ Pa}^{-1}$). Light/heavy shading denotes increases $> 7.5 \times 10^{-8} / 1.5 \times 10^{-7} \text{ K s}^{-1} \text{ Pa}^{-1}$ respectively. Note the similarity with the corresponding total ozone change (Fig. 2c).

13. WMO, *Atmospheric Ozone 1985, Global Ozone Research and Monitoring Project Report no. 16* (1985).
14. McIntyre, M. E. & Palmer, T. N. *Nature* **305**, 593–600 (1983); *J. atmos. terr. Phys.* **46**, 825–849 (1984).
15. Trenberth, K. E. & Olson, J. G. *NCAR Tech. Note NCAR/TN-301+STR* (1988); *NCAR Tech. Note NCAR/TN-299+STR* (1988).

ACKNOWLEDGMENTS. We thank Paul Fraser, Danny McKenna and Ian Watterson for discussions. R.A.P. acknowledges support from NASA. The Lauder Ozone probe program is supported by the Chemical Manufacturers Association.

An experimental observation of chaos arising from the interaction of steady and time-dependent flows

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APPLICATION of the results from studies of finite-dimensional dynamical systems to the understanding of turbulence in fluid flows is an area of great current interest. A complete description of turbulence is, however, still lacking, although new and interesting ideas continue to emerge¹. There have been some notable successes in the description of the evolution of temporal chaos in small-scale fluid-flow experiments, for example, in terms of period doubling^{2,3}. Here we present experimental results from a study of fluid motion between concentric rotating cylinders (Taylor–Couette flow). We have used laser techniques to explore the velocity field of the fluid as it proceeds from regular to weakly chaotic motion when an external control parameter is varied. We have applied modern signal-processing techniques to the velocity time series to identify temporal structures in the flow field. The evolution of these features is qualitatively the same as that found in sets of equations of much simpler form than those governing the motion of fluid flows. Our results provide striking evidence in support of the claim, as yet unproven, that there is a connection between the Navier–Stokes equations of fluid motion and properties of finite-dimensional dynamical systems.

The fluid is contained in an annular gap between two concentric cylinders. The inner cylinder and the end plates attached to it rotate⁴ and so three of the four walls containing the fluid are in motion. The radius of the inner cylinder, r_1 , is 15.87 ± 0.01 mm and that of the outer (stationary) one, r_2 , is 31.75 ± 0.01 mm, giving a radius ratio of 0.5. The dimensionless length or aspect ratio of the annular gap is $\Gamma = l/d$, where $d = r_2 - r_1$ and l is the length of the gap. An external micrometer is used to vary the aspect ratio, which typically ranges from 3 to 5 in the present arrangement.

The relevant dynamical parameter in this situation is the Reynolds number, $Re = \omega r_1 d / \nu$, where ω is the angular frequency of rotation of the inner cylinder and ν is the kinematic viscosity of the fluid. We used silicone oil, having $\nu = 17.26$ cSt at 27.3 °C, as the working fluid in all of the experiments. The entire apparatus is enclosed in an air-temperature-controlled cabinet and the cylinders are encased in a liquid-temperature-controlled bath. By these means and by the accurate control of the motor that rotates the inner cylinder, we are able to maintain the stability of Re to better than 0.1%. We have found this degree of accuracy to be essential for reproducibility of results on a variety of bifurcation phenomena, because some of the qualitative changes in the nature of the fluid motions occur for very small changes in the parameters. Furthermore, some of the flow oscillations can have long periods, so that the data are sometimes recorded over many hours.

We measure the radial velocity at a point in the fluid using

the laser Doppler technique. The apparatus may be moved vertically by a computer-controlled lift, thus enabling us either to scan the velocity profiles or to position the cylinders accurately so as to focus on a particular point of interest. The time series from the velocimeter is recorded by a Masscomp 5600 computer, on which it may be displayed or processed. We can carry out power-spectra analyses to identify specific frequency components in the signal, use statistical methods to quantify long-term recurrences and, most importantly, reconstruct phase portraits using the singular-value decomposition technique⁵. Each coordinate in the reconstructed phase space is a weighted sum of time-delayed samples of the data. The weights are calculated using statistical properties of the data in such a way as to produce a set of coordinates that are independent. We then reconstruct our 'phase portrait' by projecting the time series onto three of these coordinates which are chosen to have the maximum signal-to-noise ratio.

In the range of Re studied here, the flow occurs mainly in concentric rings around the inner cylinder, with secondary circular motion superposed in a direction orthogonal to the main azimuthal component. The fluid particles thus follow spiral paths so that a front view of a visualized flow would show fluid tori stacked upon each other. In the present case, we would expect either two or four of these tori or cells, the number depending on the control parameters Re and Γ .

The important symmetries associated with this flow are the continuous azimuthal $SO(2)$ symmetry group acting around the annulus and the discrete Z_2 mirror plane symmetry along the annular gap. In certain ranges of Re and Γ , some of the steady flows may undergo bifurcations that break the Z_2 symmetry, leading to pairs of asymmetric flows. Time-dependent flows, on the other hand, may retain the $SO(2)$ symmetry in the axisymmetric mode or break it when travelling waves are formed.

The 'phase portrait' in Fig. 1b is reconstructed from the time series of Fig. 1a, which was measured from one of a pair of time-dependent asymmetric four-cell flows. The time series shows that the flow consists of two frequency components of very different period. The higher-frequency component corresponds to a travelling tilt wave of wavenumber one and frequency $\sim 0.23\omega$, which breaks the $SO(2)$ symmetry. The other frequency has a period 30 times that of the tilt wave and is axisymmetric, that is, it is in phase at all points around the cylindrical gap.

The reconstructed 'phase portrait' shows that the motion takes place on a fat torus which has a narrow central core. The concentration of points at one end indicates that the flow spends a long time (~ 100 revolutions of the inner cylinder) near this weakly unstable fixed point, which corresponds to an asymmetric four-cell flow. The unstable direction is along the core towards the weakly attracting fixed point at the other end, which corresponds to a symmetric four-cell state. This latter point is strongly unstable with respect to motion in a plane orthogonal to the core. The instability takes the form of a path spiralling back towards the asymmetric point at the other end. The process repeats with a period of ~ 90 s. This 'phase portrait' is qualitatively similar to those found in sets of ordinary differential equations that describe the Silnikov mechanism of the onset of chaos through homoclinicity⁶.

A homoclinic orbit is defined to be a trajectory that asymptotically approaches the same fixed point as the time, $t \rightarrow \pm\infty$; this orbit will have infinite period. Also, the fixed point must have both a stable and an unstable manifold associated with it, that is, it is a saddle point. Homoclinic orbits of this type have been discussed previously in the context of fluid flows by Aubry *et al.*⁷, who investigated an ordinary-differential-equation model of boundary-layer flows derived from the Navier–Stokes equations. Silnikov⁶ studied the behaviour of the long-period orbits that exist near such points, where the motion on one of the manifolds is a spiral on a sheet and on the other is a line. This type of behaviour is consistent with that seen in the experi-

mental 'phase portrait' presented in Fig. 1*b*, where the spiralling motion is inward at one end and outward at the other with a long quiescent period in between.

Silnikov also showed that near such homoclinic points, chaotic motion can exist and may be realized by varying an external control parameter. By changing the aspect ratio by a small amount we obtain the time series and the 'phase portrait' shown in Fig. 2. The flow is now chaotic in the sense that the higher-frequency wave 'packets' no longer arrive at regular intervals. The overall structure of the 'phase portrait' is retained but the orbits join the central core at irregular points. Such

irregular homoclinic behaviour has been observed previously in a chemical oscillator⁸, but we believe that this is the first observation in a fluid flow. Although this is a weak form of chaotic motion and is, of course, far from turbulence, the results are very encouraging in that it seems possible to describe the motion of this infinite-dimensional system in its regular phase by a relatively simple set of finite-dimensional ordinary differential equations, given in chapter 7 of ref. 6. Moreover, these phenomena have been found at low Reynolds numbers, where the basic bifurcation structure is understood from our numerical work⁹. Theoretical progress could therefore be made by lineariz-

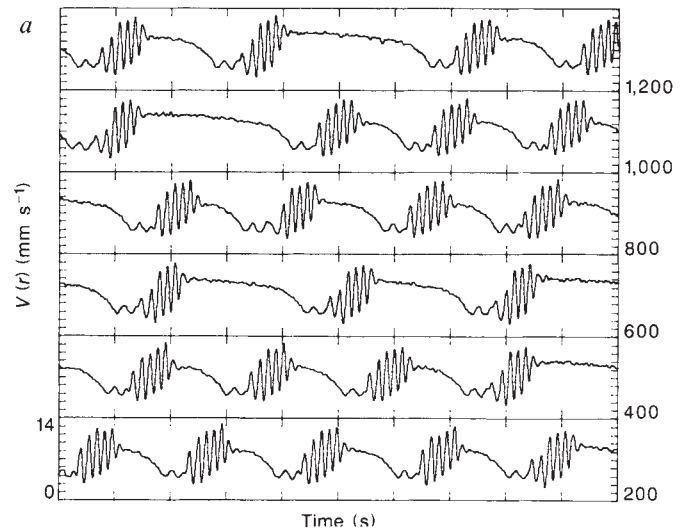
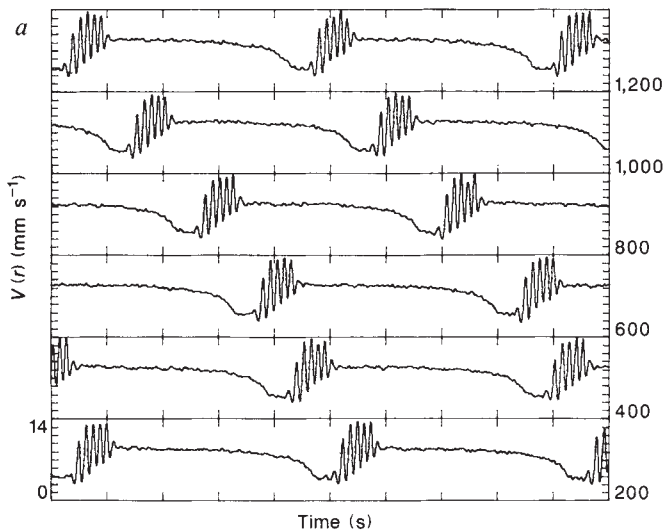


FIG. 1 *a*, Time series of radial velocity component of the flow measured at mid-gap of the annulus in the regular regime; $Re=129.47$, $\Gamma=4.278$. *b*, 'Phase portrait' reconstructed from the time series of *a*. See text for details.

FIG. 2 *a*, Time series of radial velocity component of the flow measured at mid-gap of the annulus in the irregular regime; $Re=129.47$, $\Gamma=4.272$. *b*, 'Phase portrait' reconstructed from the time series of *a*.

ing the equations of motion about these known solutions to produce a finite-dimensional model which would establish a direct link between theory and experiment, thus permitting one to investigate numerically the onset of chaos. □

Received 7 April; accepted 14 June 1989.

1. Argoul, F. *et al. Nature* **338**, 51–53 (1989).
2. Libchaber, A., Laroche, C. & Fauve, S. *J. Phys., Paris* **43**, L211–L216 (1983).
3. Pfister, G. *Lecture Notes in Physics* **235**, 199–210 (Springer, Berlin, 1985).
4. Tavener, S. J., Mullin, T. & Cliffe, K. A. *J. Fluid Mech.* (in the press).
5. Broomhead, D. S. & King, G. P. *Physica D* **20**, 217–235 (1986).
6. Guckenheimer, J. & Holmes, P. *Nonlinear Oscillations, Dynamical Systems and Bifurcations of Vector Fields* (Springer, Berlin, 1983).
7. Aubry, N., Holmes, P., Lumley, J. L. & Stone, E. *J. Fluid Mech.* **192**, 115–173 (1988).
8. Roux, J. C., Rossi, A., Bachelart, S. & Vidal, C. *Physica D* **2**, 395–403 (1981).
9. Mullin, T., Tavener, S. J. & Cliffe, K. A. *Europhys. Lett.* **8**, 251–256 (1989).

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A direct link between the China loess and marine $\delta^{18}\text{O}$ records: aeolian flux to the north Pacific

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MOST studies of Quaternary climates conducted during the past 150 years have focused on glacial sequences, and for the past 25 years marine sediments have provided significant further understanding of glacial and interglacial palaeoclimates. An important goal of palaeoclimatology, however, to link the well-known detailed continental records to marine records of similar resolution, has yet to be realized. The classic loess sequences of China provide an apparently continuous record of continental climate over the past 2.4 million years^{1,2}. The oxygen isotope, $\delta^{18}\text{O}$, timescale developed from the marine record³ gives us a global chronostratigraphy of late Pleistocene climates. Previously, it has been suggested in studies of the aeolian component in pelagic sediments that it may be possible to link these two records directly by finding a suitable core from the north-west Pacific downwind from China^{4,5}. Here we report the results of such an effort for core V21-146 (Fig. 1) and tie the last 500,000 years of the Chinese loess–soil sequence directly to the $\delta^{18}\text{O}$ timescale and, in so doing, refine the dating of regional loess and soil horizons.

The advantage of using oceanic sediments to infer palaeoclimatic conditions of eastern Asia is the reliability and uniformity of the dating techniques for marine sediments. Late Pleistocene variations in $\delta^{18}\text{O}$ values in the CaCO_3 of benthic foraminifera shells reflect the changing oxygen-isotope composition of the oceans, which is primarily determined by the volume of isotopically light continental glacial ice⁶—the more ice, the greater the ^{18}O concentration in the oceans. The Specmap project³ has defined the timing of $\delta^{18}\text{O}$ variations in the ocean of the past 800 kyr, providing the basic chronostratigraphy for late Pleistocene palaeoclimatology. Furthermore, since the late Pleistocene $\delta^{18}\text{O}$ record is a measure of ice volume, palaeoclimatological indications found in oceanic sediments can be directly compared to presumed glacial–interglacial cycles anywhere on Earth.

TABLE 1 Basal age estimates for Xifeng loess sequence.

Unit	Depth (m)	Susceptibility model age ⁹ (kyr)	Aeolian age (kyr)
S0	1.0	11	12
L1	LL1 4.4	27	33
	SS1 8.2	56	50
	LL2 11.4	71	85
S1	14.2	126	120
	LL1 16.4	138	151
	SS1 18.2	147	172
L2	LL2 20.2	157	187
	SS2 20.8	161	197
	LL3 22.4	173	209
S2	SS1 24.2	216	229
	LL1 26.4	230	244
	SS2 27.8	251	259
L3	30.9	271	291
	SS1 32.0	290	300
	LL1 32.8	302	310
S3	SS2 34.2	331	336
	39.4	361	363
S4	42.6	432	427
	46.8	454	458
	SS1 48.0	463	468
L5	LL2 49.2	472	476

Loess units and depths from ref. 9.

Many palaeoclimatic studies of eastern Asia have been based on loess^{1,2,7}, a terrestrial windblown silt deposit⁷. Loess, the most extensive Quaternary sediment in central and northern China, covers over 440,000 km², often to depths greater than 200 m. These sequences typically consist of layers of silty loess interbedded with weathered loess or palaeosols. Although these alterations have been ascribed to fluctuating palaeotemperatures or weather patterns^{1–8}, pedological evidence suggests a closer relationship to the effective soil moisture which controls the nature and density of vegetational cover⁹. Thus, the alternating sequence of loess and soil layers is an indicator of regional precipitation which records oscillations between relatively arid and more humid conditions.

To tie the China loess sequence to other regional and global records of Quaternary climate change, it is important to develop an accurate chronology for the sequence and, if possible, link it to the marine $\delta^{18}\text{O}$ record of palaeoclimate. Unfortunately most terrestrial sequences are difficult to date; biostratigraphic and early magnetostratigraphic investigations proved erroneous and unreliable. Recent advances in thermoluminescence,

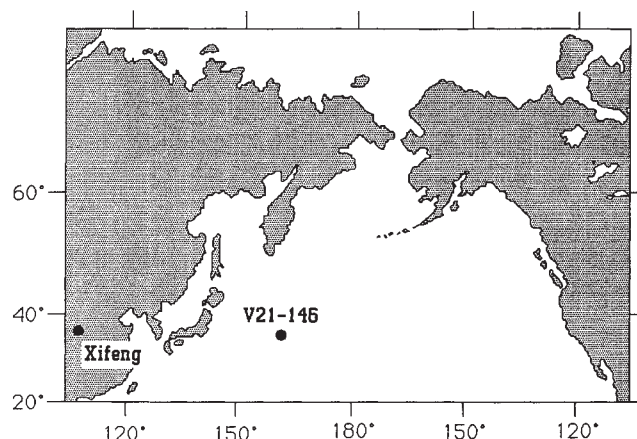


FIG. 1 Map showing the location of Xifeng loess sequence and core V21-146.

palaeomagnetic and radiocarbon techniques have greatly improved dating reliability¹⁰, yet an accurate, high-resolution chronostratigraphy has not been developed. Perhaps the most promising approach is based on the magnetic-susceptibility measurements of loess deposits by Kukla¹¹, who found that the magnetic susceptibility of loess and interbedded soils varies with the concentration and the grain size of magnetic minerals and increases with the degree of pedogenesis. Kukla assumed a constant deposition rate for magnetic minerals, interpolated between palaeomagnetic reversal horizons and devised a time-scale based on sediment thickness, weighted by susceptibility (Table 1; Fig. 2).

The mass accumulation rate of the aeolian component of oceanic sediments is an indicator of continental climate⁵, as shown by the observation that the amount of dust blowing across the north Atlantic is related to the climatic conditions in the Sahara-Sahel source region¹². Greater annual fluxes of aeolian material occur when conditions are more arid in North Africa¹³. In the north Pacific away from areas of river input and ice rafting, surface sediment mineralogy patterns match those of atmospheric aerosols¹⁴⁻¹⁷, suggesting that wind transport is the primary input mechanism. Pleistocene records of aeolian deposition in the north Pacific contain Milankovitch orbital periodicities^{4,18} while longer-term records of aeolian deposition in the region have revealed important changes in aeolian deposition since the Late Cretaceous period^{5,19-21}. These studies suggest that aeolian dust composes essentially the entire mineral fraction of the north Pacific pelagic sediments and is a valuable indicator of source-region climate.

To tie the loess sequence of China to the $\delta^{18}\text{O}$ chronostratigraphy and palaeoclimatic record we have used core V21-146

(37° 41' N, 163° 02' E, 3,968 m; Fig. 1) which provides a continuous record of pelagic sedimentation in the north-west Pacific during much of the late Pleistocene epoch. This site is approximately 2,500 km downwind from mainland China and 3,500 km from the important dust sources in central China² (Fig. 1). An oxygen isotope stratigraphy determined from benthic foraminifera was matched to the Specmap standard³ to develop a detailed chronostratigraphy for this core. An average linear sedimentation rate of $\sim 3.0 \text{ cm kyr}^{-1}$ and a sampling interval of 10 cm provided temporal resolution of $\sim 3\text{--}4 \text{ kyr}$ (Fig. 2). The aeolian component of each sample was isolated by a sequence of chemical extractions²². Since this method does not remove volcanogenic components, visual estimates were made from smear slides of ash in the extracted samples and these percentages subtracted from the total amount of extracted material to give true abundance values of continentally derived dust. To avoid the masking of any palaeoclimatic signal because of dilution by other, commonly biogenic, sedimentary components, dust fluxes are expressed as mass accumulation rates $\text{mg cm}^{-2} \text{ kyr}^{-1}$.

The formation of mid-latitude loess occurs during the glacial periods of the Plio-Pleistocene epochs^{7,10}. Since loess forms in semi-arid conditions during times of glaciation⁹, maximum fluxes of aeolian dust to the north-west Pacific should coincide with late Pleistocene glaciations⁵. This is the pattern we have found in V21-146 with flux maxima at about 28, 69, 137, 185, 271, 343 and 473 kyr (Fig. 3). Using Kukla's ages², we observe a correlation between the deposition of loess layers in China and greater accumulation of aeolian material in the deep sea. The gradual increase of oceanic aeolian fluxes over the past 500 kyr (Fig. 3) apparently reflects a trend toward greater aridity

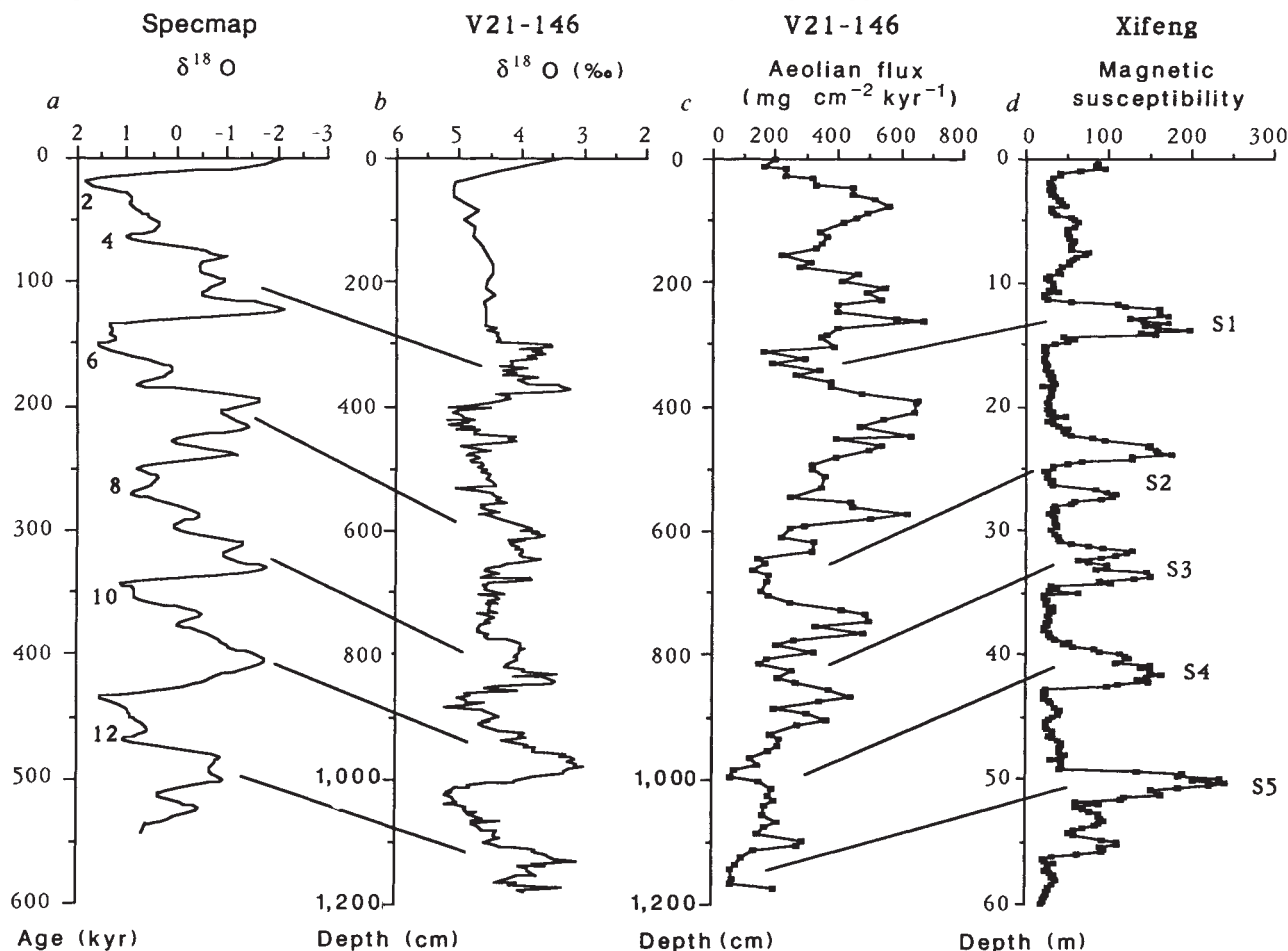


FIG. 2 *a*, Specmap oxygen isotope standard³. *b*, Oxygen isotope stratigraphy for V21-146. *c*, Detailed stratigraphy of aeolian deposition in the north Pacific for the past 530 kyrs. *d*, The magnetic susceptibility of the Xifeng

loess sequence, which displays an approximate inverse correlation to North Pacific aeolian flux with high-susceptibility palaeosols coinciding with reduced rates of dust deposition in V21-146.

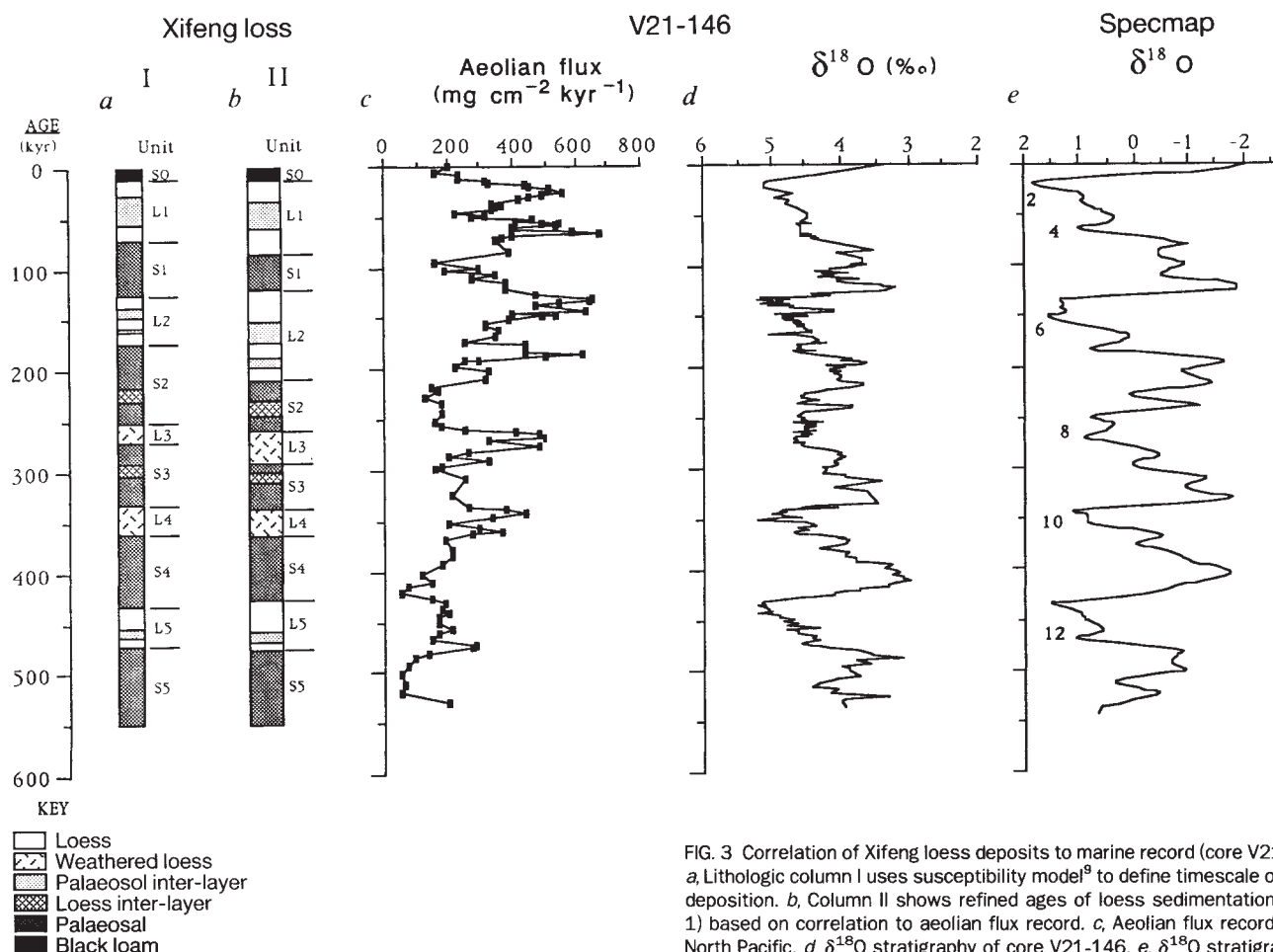


FIG. 3 Correlation of Xifeng loess deposits to marine record (core V21-146). *a*, Lithologic column I uses susceptibility model⁹ to define timescale of loess deposition. *b*, Column II shows refined ages of loess sedimentation (Table 1) based on correlation to aeolian flux record. *c*, Aeolian flux record of the North Pacific. *d*, $\delta^{18}\text{O}$ stratigraphy of core V21-146. *e*, $\delta^{18}\text{O}$ stratigraphy of Specmap global record³.

of the climate system in eastern Asia during the late Pleistocene epoch. Similar inferences have been drawn by Pye and Li⁸ from observations of increased rates of dust accumulation in the Xifeng loess sequence.

The high-quality aeolian record observed in V21-146 can be linked directly to the loess-soil sequence in Xifeng⁹ and to the $\delta^{18}\text{O}$ timescale³ (Fig. 3). Combining the accuracy for the $\delta^{18}\text{O}$ timescale (3–5 kyr) (ref. 3) with the temporal resolution in V21-146 of 3–5 kyr allows age estimates of loess horizons to within ± 6 kyr. If we then refine loess-soil ages on the basis of the aeolian correlation model (Table 1), the age span of loess layers L1 and L2 is expanded and the age span of soils S1 and S2 is reduced. Only minor changes are made to older horizons, and in general, the agreement between age assignments made on the basis of the susceptibility model and on our aeolian correlation model is good (Fig. 3). □

19. Janecek, T. R. & Rea, D. K. *Geol. Soc. Am. Bull.* **94**, 730–738 (1983).
20. Janecek, T. R. *Init. Rep. DSDP Burcklo, L. H.* **86**, 589–603 (1985).
21. Leinen, M. *Init. Rep. DSDP Burcklo, L. N.* **86**, 581–588 (1985).
22. Rea, D. K. & Janecek, T. R. *Init. Rep. DSDP Vallier, T. L.* **62**, 653–659 (1981).

ACKNOWLEDGEMENTS. We thank G. Kukla for tabulations of his magnetic susceptibility data and comments on this manuscript. Samples from V21-146 were provided by the Core Laboratory of Lamont–Doherty Geological Observatory. This work was supported by NSF, Climate Dynamics Program.

High-temperature hydrothermal precipitation of precious metals on the surface of pyrite

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A KNOWLEDGE of high-temperature surface chemistry processes is essential to a complete understanding of the genesis of hydrothermal gold deposits and is also of considerable importance to industrial mineral processing. Here we have studied individual pyrite grains separated from four lode-type gold deposits using optical and scanning electron microscopy and looked at the textural relationships of late gold on the surface of the pyrite. The gold occurs as sub-spherical to subhedral, rarely euhedral, grains. In three of the deposits studied, it is variously accompanied by native tellurium, native silver and altaite. Discrete crystals of gold and

Received 3 April; accepted 16 June 1989.

1. Liu, T. S. et al. *Loess and the Environment* (Ocean, Beijing, 1985).
2. Kukla, G. *Quat. Sci. Rev.* **6**, 191–219 (1987).
3. Imbrie, J. et al. in *Milankovitch and Climate, Understanding the Response to Orbital Forcing, Part 1* (eds Berger, A., Imbrie, J., Hays, J., Kukla, G. & Saltzman, B.) 269–305 (Reidel, Dordrecht, 1984).
4. Janecek, T. R. & Rea, D. K. *Quat. Res.* **24**, 150–163 (1985).
5. Rea, D. K., Leinen, M. & Janecek, T. R. *Science* **227**, 721–725 (1985).
6. Shackleton, N. J. *Nature* **218**, 15–17 (1967).
7. Pye, K. *Aeolian Dust and Dust Deposits* (Academic, London, 1987).
8. Pye, K. & Li, P. Z. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (in the press).
9. Kukla, G. & An, Z. S. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (in the press).
10. Heller, F. & Liu, T. S. *Nature* **300**, 431–433 (1982).
11. Kukla, G. et al. *Geology* **16**, 811–814 (1988).
12. Prospero, J. M., Giaccum, R. A. & Nees, R. T. *Nature* **289**, 570–572 (1981).
13. Prospero, J. M. & Nees, R. T. *Nature* **320**, 735–738 (1986).
14. Ferguson, W. S., Griffin, J. J. & Goldberg, E. D. *J. geophys. Res.* **75**, 1137–1139 (1970).
15. Leinen, M. & Heath, G. R. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **36**, 1–21 (1981).
16. Blank, M., Leinen, M. & Prospero, J. M. *Nature* **314**, 84–86 (1985).
17. Uematsu, M., Duce, R. A. & Prospero, J. M. *J. Atmos. Chem.* **3**, 123–138 (1985).
18. Pisias, N. G. & Leinen, M., in *Milankovitch and Climate, Understanding the Response to Orbital Forcing, Part 1* (eds Berger, A., Imbrie, J., Hays, J., Kukla, G. & Saltzman, B.) 307–330 (Reidel, Dordrecht, 1984).

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the other metals are located at specific sites on the pyrite substrate, showing a marked affinity for crystal edges and surface defects. These observations confirm the importance of the conducting properties of sulphides to metal precipitation. We believe that adsorption–chemisorption processes are particularly effective at point defects in the crystal surfaces and that these were responsible for the destabilization of aqueous Au-thio-sulphide, Au-thio-telluride, and Ag-bearing complexes, resulting in the precipitation of the gold, tellurium, and silver.

The mineralogical associations of gold were investigated from four different types of lode gold deposits currently being studied at Southampton: Gebeit Mine, Sudan—a Proterozoic vein deposit in a complex shear-thrust zone; Lennox Mine, Zimbabwe—lensoid quartz veins adjacent to an Archaean iron formation¹; Dalny Mine, Zimbabwe—an Archaean ductile–brittle shear zone; Golden Valley Mine, Zimbabwe—an Archaean quartz vein. In these four deposits, pyrite occurs as idiomorphic grains (0.3–1.0 mm) and as polycrystalline aggregates. In hand specimens, visible gold is closely associated with pyrite and also occurs as disseminated coarse grains and fracture-controlled gold-only stringers in sulphide-poor quartz. Arsenopyrite is a common component of the Dalny and Gebeit orebodies, and often displays evidence of brittle fracturing. High-grade ore samples of vein quartz that contained sulphides were selected for hydrofluoric acid digestion of the quartz matrix ($\pm$ carbonate $\pm$ chlorite $\pm$ tourmaline) using established palynological dissolution techniques², which reportedly do not result in etching of the pyrite³. Our subsequent observations confirmed the lack of surface dissolution. The residual gold and sulphide grains were examined under a binocular microscope and specific grains individually mounted for examination by scanning electron microscopy and electron diffraction spectroscopy to assess the micro-structural features of the gold and its inter-relationships with the sulphides.

In all four deposits, the gold occurs as native grains adhering to the surface of pyrite grains (Figs 1*a, b*). The habit of the native metal varies from good crystalline form (Fig. 2*a*), to subhedral grains (Fig. 2*b*), and to sub-spherical and domal grains (Fig. 1*b*). Many of the gold grains developed on crystal faces display flattened surfaces at the gold–pyrite interface (Figs

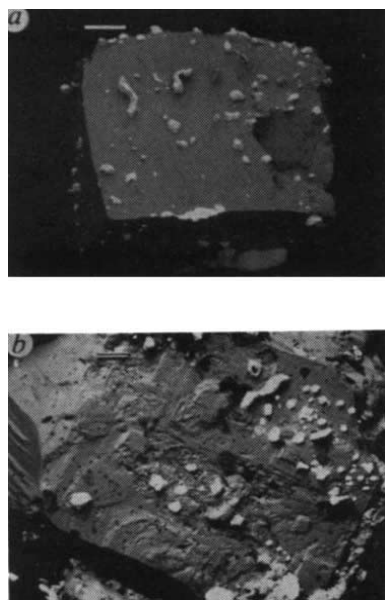


FIG. 1 Back-scattered electron (BSE) images of gold and telluride on pyrite. *a*, Gold and altaite on pyrite crystal faces and edges, Lennox Mine; scale bar = 100 μm . *b*, Gold on hydrothermally etched pyrite, Golden Valley Mine; scale bar = 100 μm .

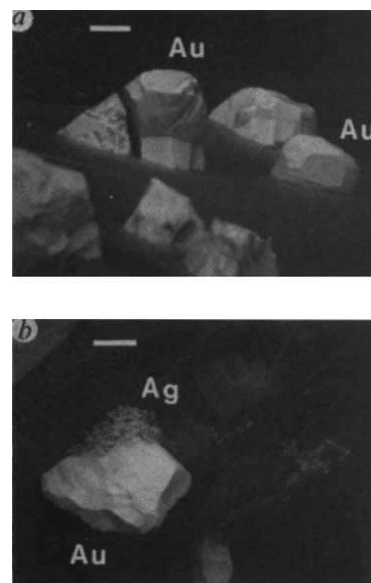


FIG. 2 Precious metals and altaite on pyrite, Lennox Mine. *a*, Higher magnification image of the right-central portion of Fig. 1*a*; gold and altaite grains displaying planar basal surfaces adjacent to the pyrite; BSE image, scale bar = 10 μm . *b*, Gold and filigree silver on pyrite; BSE image, scale bar = 20 μm .

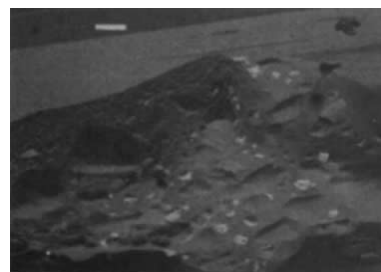


FIG. 3 Fine crystalline aggregates of gold and tellurium (white) on a fractured surface of pyrite, Golden Valley Mine; BSE image, scale bar = 20 μm .

2*a, b*). The planar nature of this interface has been confirmed by sequential H_2O_2 leaching of individual pyrite grains which removed successive thin layers (1–10 μm) of iron sulphide and readily liberated the gold.

The morphology of the substrate, pyrite, ranges from the simple cube $a\{100\}$ through to partial development of the octahedron $o\{111\}$, which is indicative of crystal growth under conditions of low to moderate degrees of supersaturation⁴. Where good planar pyrite crystal faces have been developed, there is no evidence for any crystallographic control of the distribution of the gold, although it is occasionally concentrated along crystal edges (Fig. 1*a*). Other pyrite crystals exhibit a preferential concentration of gold on fracture surfaces (Fig. 3) and surfaces where hydrothermal etching has partially destroyed cleavage faces (Fig. 1*b*).

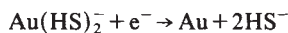
Our study revealed that the tellurium-bearing phases are relatively common, though minor, minerals in Lennox and Golden Valley, and are present less commonly in the Dalny deposit, although they have not previously been reported from these environments. Altaite (PbTe) and hessite (Ag_2Te) occur as discrete crystals (Fig. 2*a*) and both native tellurium and native silver are evident at Lennox. The native metals occur both as spongiiform aggregates and as delicate filigree growths in contact with the pyrite (Fig. 4). Filigree silver also mantles late gold (Fig. 2*b*). Native tellurium is a minor but integral part of the

mineralization at Golden Valley, occurring as small tabular crystals and infilling etch pits in pyrite (Fig. 3).

Our observations indicate that a significant proportion of the gold in all four deposits examined was precipitated after crystallization of the iron-bearing sulphides and even after the vein quartz. In three of the four deposits, this late gold was accompanied or even post-dated by precipitation of native silver and native tellurium. The metal precipitation at the surface of the pyrite took place either directly from the hydrothermal fluid during a hiatus in quartz precipitation or during later fluid over-pressuring of the quartz-pyrite intergrowth which permitted ingress of the fluid into micro-dilatational envelopes surrounding the pyrite. The euhedral form of some gold crystals is indicative of precipitation from fluids which, at most, were only slightly supersaturated with gold. Conversely, the filigree textures of some of the silver and tellurium clearly suggest precipitation of the metals from highly supersaturated solutions in locally unstrained environments. The preservation of the delicate crystal forms also indicate the absence of any later superimposed strain. Although showing a generally random distribution, it is evident that precipitation of the gold and other metals was focused at specific nucleation points on the crystal faces, face edges, and fracture surfaces of the pyrite. There is no evidence for thin-skin mantling of complete surfaces.

Metamorphic recrystallization of auriferous pyrite and diffusion of gold through the pyrite lattice⁵ is discounted as the gold rarely displays any coherent distribution on the pyrite surfaces that might be construed as having occurred through crystallographically controlled diffusion and exsolution. Furthermore, terraced crystal faces, indicative of the layer-by-layer primary growth mechanism of the pyrite⁴, have not been annealed by subsequent metamorphic event(s) and the native metals and tellurides often mantle multiple terraces (Fig. 4). Similarly, the destabilization of gold thio-sulphide complexes due to interaction of the aqueous species with mineral-hosted ferrous iron^{6,7} is discounted due to the lack of any evidence for secondary iron sulphides intergrown with the late precious metals.

Precipitation of gold on sulphide surfaces has been demonstrated experimentally⁸⁻¹¹ and our observations support the contention of other workers that the conductivity of the sulphide surfaces plays an important role in the gold-precipitation¹⁰⁻¹³. Pyrite, in common with a number of other sulphide minerals, is a semiconductor, exhibiting a conductivity intermediate between that of insulators and that of metals. As a semiconductor, it exhibits two types of behaviour: n-type in which the carriers (defects) contain additional electrons and p-type in which electrons have been removed and holes are dominant¹⁴⁻¹⁶. Gold tends to be adsorbed predominantly by p-type and mixed n-p-type pyrite^{12,13}; p-type behaviour in pyrite can be induced by the presence of acceptor cations such as arsenic¹⁵. We believe that a two-stage process, somewhat analogous to that of Bancroft and co-workers^{10,11}, occurs at the pyrite-electrolyte interface. This involves physical adsorption of the charged aqueous gold species (probably a thio species^{17,18}) followed by reduction-driven chemisorption of the gold species to precipitate the native gold:



Our observations did not reveal a widespread gold plating of the pyrite analogous to that generated by the low-temperature experimentation of Hyland and Bancroft¹¹. Consequently, we suggest that maximum conductivity and hence polarization was associated with small physical defects (for example, fractures, etch pits and negative crystal moulds) on the crystal surfaces and along the crystal edges, which enhanced physical adsorption of the charged species and the enveloping layer of oriented water molecules¹⁶. The disruption of surface bonding in the same defects would then lead to enhanced chemical bonding of the ionic species, both effects thus accounting for the site-

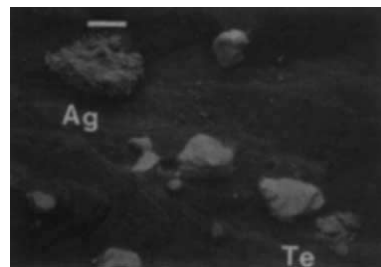


FIG. 4 Altaite, silver, tellurium, and fine native gold mantling multiple terraces on pyrite, Lennox Mine. The silver has filigree form and the tellurium is predominantly spongiform; BSE image, scale bar, 20 μm .

dependent distribution of the gold. The high electrical conductivity of the gold would ensure transmission of the electronic characteristics of the nucleation site to the gold-fluid interface, thus facilitating the early growth of individual crystals¹⁰. The ability of p-type pyrite to collect electrons is finite, however, and thus the continued growth of the gold must eventually have been due to the presence of structurally favourable epitaxial sites in the new gold substrate.

Any precipitation mechanism also has to take into account the common occurrence of altaite and less common co-precipitation of silver and tellurium evident in some deposits. The presence of native tellurium in three of the deposits indicates that an exceptionally high tellurium fugacity, f_{Te} , prevailed in the late fluid¹⁹. Furthermore the occurrence of discrete tellurium and telluride minerals on pyrite suggests that the two-stage adsorption-chemisorption process was also responsible for destabilization of a gold thio-telluride complex and the consequent synchronous precipitation of gold and tellurium. Silver is transported either as an aqueous chloride²⁰ or thio-complex²¹ and thus a similar mechanism may account for precipitation of the silver.

We have described some characteristics of late precious-metal assemblages associated with pyrite in lode-gold deposits. The occurrence of the metals along crystal edges and nucleated on and within specific crystal defects clearly points to the important role of pyrite as a good conductor and to its contribution to the destabilization of aqueous complexes of gold and other metals by adsorption-chemisorption processes. Other metal sulphides are equally good conductors and undoubtedly act as suitable substrates for gold precipitation, but the abundance of pyrite in most lode-gold deposits emphasizes the important contribution of pyrite-electrolyte interactions to gold metallogenesis. $\square$

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- Gilligan, J. M. & Foster, R. P. *African Mining* (Instr. Min. Met.), 127-138 (1987).
- Phipps, D. & Playford, G. *Dept. Geol., Univ. Queensland* **11**(1) (1984).
- Neuerburg, G. J. *Am. Miner.* **46**, 1498-1501 (1961).
- Murowchick, J. B. & Barnes, H. L. *Am. Miner.* **72**, 1241-1250 (1987).
- Boyle, R. W. *The Geochemistry of Gold and its Deposits* (Geological Survey of Canada, Ottawa, 1979).
- Phillips, G. N., Groves, D. I. & Martyn, J. E. *Econ. Geol.* **79**, 162-171 (1983).
- Neall, F. B. *Geol. Dept. Univ. Ext., Univ. Western Austr. Pub.* **11**, 265-269 (1987).
- Sakharova, M. S., Batrakova, Yu. A. & Ryakhovskaya, S. K. *Geochem. Int.* **12**, 84-89 (1975).
- Sakharova, M. S., Batrakova, Yu. A. & Ryakhovskaya, S. K. *Geochem. Int.* **13**, 160-166 (1976).
- Jean, G. E. & Bancroft, G. M. *Geochim. cosmochim. Acta* **49**, 979-987 (1985).
- Hyland, M. M. & Bancroft, G. M. *Geochim. cosmochim. Acta* **53**, 367-372 (1989).
- Mironov, A. C., Zhmodik, S. M. & Maksimova, E. A. *Geochem. Int.* **18**, 153-160 (1981).
- Colvine, A. C. et al. *Ontario. Geol. Surv. Open File Rep.* 5524 (1984).
- Smith, F. G. *Am. Miner.* **27**, 1-19 (1942).
- Pridmore, D. F. & Shuey, R. T. *Am. Miner.* **61**, 248-259 (1976).
- Shuey, R. T. *Semi-conducting Ore Minerals* (Elsevier, Amsterdam, 1975).
- Seward, T. M. *Geochim. cosmochim. Acta* **37**, 379-399 (1973).
- Shenberger, D. M. & Barnes, H. L. *Geochim. cosmochim. Acta* **53**, 269-278 (1989).
- Afifi, A. M., Kelly, W. C. & Essene, E. J. *Econ. Geol.* **83**, 377-394 (1988).
- Seward, T. M. *Geochim. cosmochim. Acta* **40**, 1329-1341 (1976).
- Gammons, C. H. & Barnes, H. L. *Geochim. cosmochim. Acta* **53**, 279-290 (1989).

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Experimental evidence for very low solubility of rare-earth elements in CO₂-rich fluids at mantle conditions

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CHEMICAL compositions of mantle-derived magmas and mantle xenoliths suggest that some regions of the mantle were enriched in basaltic components (CaO, FeO, Al₂O₃ and alkalis), large-ion lithophile elements, or both before melting or disruption and entrainment¹⁻⁶. Some peridotite xenoliths that are depleted in basaltic components contain clinopyroxenes with chondrite-normalized La/Yb ratios of >30 and La contents of 100-times chondritic. Other xenoliths with similar major-element chemistry contain clinopyroxenes with La_N/Yb_N=0.1-1 and [La]=1-10-times chondritic (reviewed in ref. 7) Enrichment in light rare-earth elements without concomitant increase in basaltic components has been called 'cryptic metasomatism'⁸. Here we present the results of experiments at high temperature and pressure, which show that CO₂-rich fluids, the postulated agents of cryptic metasomatism⁹, dissolve only minor amounts of rare-earth elements, and cannot therefore be responsible for such enrichments.

Mysen¹⁰ determined concentrations of samarium in diopsides equilibrated with CO₂-rich fluid at 20-30 kbar, 900-1,100 °C. Values of the distribution coefficient for samarium between CO₂

and diopside, $K_D^{CO_2/Di(Sm)}$, were estimated as 5-58 by assuming that all samarium that was not in diopside had entered the fluid. These estimates and their comparison with values of $K_D^{H_2O/Di}$ for other rare-earth elements¹¹ resulted in a belief that carbonic fluids in the mantle have elevated contents of rare-earth elements and are light-rare-earth-element enriched, in agreement with estimates of the partitioning of rare-earth elements between CO₂ and liquid¹². Other experimental studies (reviewed in ref. 13) show that the solubilities of major elements in fluids decrease precipitously with increase in $X_{CO_2}^{fluid}$. These results led to a belief that carbonic fluids are excellent agents for cryptic metasomatism. Alternative models¹⁴⁻¹⁷ suggest that the reaction of carbonated silicate melts^{14,15} or carbonatite melts^{16,17} with peridotite may cryptically metasomatize the latter.

Our objective was to determine contents of neodymium in coexisting diopside-rich solid and CO₂-rich fluid at 10-15 kbar, 1,000-1,200 °C. We determined 1-atm growth rates of diopside from glass in the presence of CO₂ to estimate the required experimental run times. These results are summarized in Fig. 1 and are compared with the diameter of a diopside grain that may be homogenized by diffusion—the maximum period before attainment of equilibrium. To reduce experimental run times to reasonable lengths, it was necessary to limit final diopside grain size. Diopside glass was, therefore, ground to ≤5 μm, and the glass pieces were coated in silica (SiO₂) powder of sub-micron grain size. The weight ratio of diopside glass to SiO₂ powder was 2:1 in all these experiments (Table 1). The SiO₂ rapidly crystallized to quartz and prohibited growth of diopside grains exceeding 5 μm in size. Some diopside grains developed a narrow connection and created 'dumbbell'-shaped crystals, but most remained discrete.

Experiments were performed in a high-pressure solid-media apparatus¹⁸. Diopside glass, doped with Nd and coated in SiO₂ powder, in a crimped but not welded inner platinum capsule, was equilibrated with CO₂, which was produced *in situ* from PdO and graphite in a welded outer platinum capsule. PdO and C were added in the correct proportions to produce CO₂ with no excess C or O₂. The pressure cells, constructed of NaCl, Pyrex, graphite and alumina, were kept at 200 °C until immediately before an experiment, to ensure there was no source, such as H₂O, to release H₂ which could diffuse into the capsules and dilute the pure CO₂. If any H₂ did enter the capsule, the resultant fluid would lie on the CO₂-H₂ mixing line (Fig. 2). Only a small amount of H₂ could be added before attainment of graphite saturation, where the fluid is 87 mol % CO₂. Because our runs did not precipitate graphite, we are confident that fluids contained over 87% CO₂.

One possible problem anticipated in solid-fluid experiments is the inability of the fluid to infiltrate along mineral grain edges^{19,20}. Another series of three experiments were performed in which a calcite liquid, doped with Nd and potassium (to preserve charge balance) in the inner capsule was equilibrated with CO₂ in the outer capsule. In these experiments, the fluid had direct access to the liquid surface and no infiltration of a solid was required for attainment of equilibrium. The results of both of these sets of experiments are very similar (Table 1), implying that the fluid and condensed phases were able to equilibrate in both sets of experiments.

After quenching, the outer capsules were cleaned of ceramic, washed in hydrofluoric and nitric acids, and weighed. The capsules were opened in a Class 100 clean laboratory and weighed after opening to check the mass of CO₂ present in the capsule at run conditions. The inside of the outer capsule was leached with carbonic acid; in three replicate runs, the leachate was hydrochloric and nitric acids. The acids were decanted and the procedure repeated twice. A known amount of ¹⁴⁵Nd-isotopically-enriched spike was added. The material in the inner capsule was dissolved in nitric and hydrofluoric acids after addition of ¹⁴⁵Nd-isotopically enriched spike. Aliquots of starting materials were treated in the same manner. After dissolution and evapor-

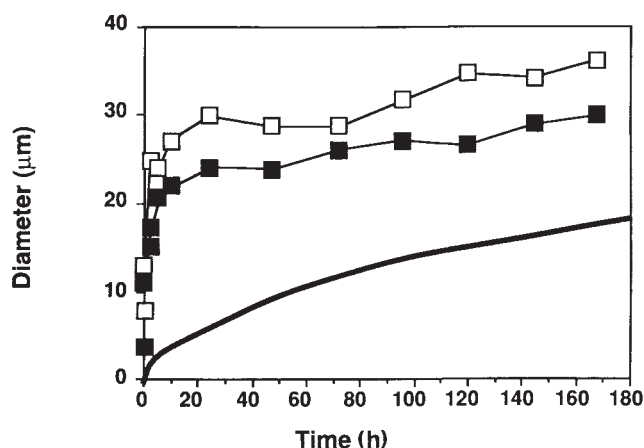


FIG. 1 The size of diopside grains against their homogenization time. The grains were crystallized from glass in the presence of CO₂ fluid in experiments with a range of run times. The time required to homogenize a diopside grain by solid-state diffusion was calculated using a model that assumes a spherical grain with a constant content of tracer phase²⁵ at the surface and using a diffusion coefficient of $3 \times 10^{-13} \text{ cm}^2 \text{ sec}^{-1}$. This is, presumably, the maximum time required before attainment of equilibrium as it assumes that Nd trapped in the centers of diopside grains must move to the grain edge by volume diffusion. The closed symbols denote the mean diameter of 30-40 grains measured; the open symbols denote the maximum diameter determined in those grains. The heavy stippled line shows the size of a grain that can be homogenized by spherical diffusion during the duration of the experiment. The extrapolated curve indicating the size of a grain that can be homogenized by diffusion and the curves denoting the actual size of the grains only intersect after 3-6 months.

ation, the residue was dissolved in hydrochloric acid and Nd was separated from other cations by standard cation-exchange techniques.

The measured $^{145}\text{Nd}/^{144}\text{Nd}$ ratio was used to determine the amount of Nd in the initial and final solid materials and the quench residue from the fluid (Table 1; Fig. 3). In all cases, the contents of Nd in the quench from the fluid were below detection limit (~ 500 pg). The final solids had contents of Nd very similar to those in the starting materials. The differences in Nd content between the initial and final solid materials for runs using diopside glass with higher contents of Nd are $\pm 2\%$ (similar to the analytical uncertainty). The differences for runs with lower contents of Nd are $\sim 5\%$, indicating some loss of Nd. We believe this is the result of an incomplete recovery of the solid, rather than from dissolution of Nd in the fluid, because no Nd was found in the outer capsule.

Secondary Nd L α X-ray photons emitted in an electron microprobe by solid products from identical runs to those listed in Table 1 were integrated for 900 s. Analysis at most grain boundaries produced no Nd emission and, therefore, low contents of Nd. A few small grains with high Nd content were found and these are believed to contain much of the Nd in the run products. These grains also have high contents of silicon but low contents of calcium and magnesium suggesting that they may be a Nd-silicate, but they are too small for quantitative analysis. Apparently, the diopside (and the silica) and the fluid were saturated in Nd. The excess Nd, lacking a suitable host phase, formed a Nd-silicate that occupied interstitial sites in the diopside-silica solid assemblage.

Two approaches for calculating the partitioning of Nd between the solid and the fluid are used. The more conservative approach is to assume that Nd not recovered from the inner capsule was present in the outer capsule but was not removed by leaching. As recovery was no worse than 95% (Table 1), apparent values of $D^{\text{CO}_2/\text{solid}}(\text{Nd})$ are <0.05 for our runs (where

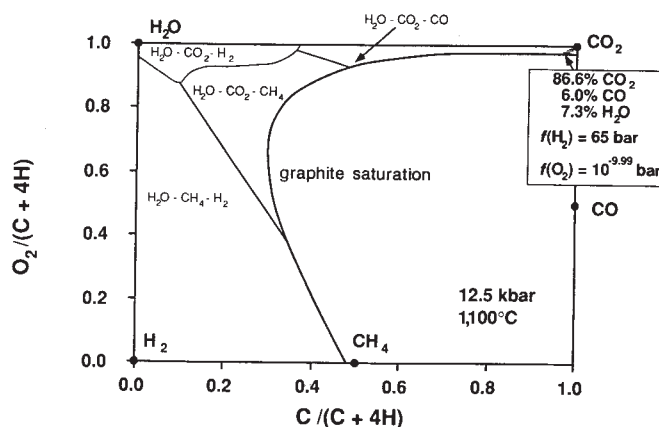


FIG. 2 Fluid compositions in molar proportions at the average of the run conditions reported in this paper, calculated from modified Redlich-Kwong equations²⁶. The fields denote the major fluid species present. The horizontally ruled line emanating from the CO_2 point is a mixing line towards H_2 . The intersection of this line and the graphite saturation surface gives the most CO_2 -poor fluid that could be produced by diffusion of H_2 into a CO_2 -containing capsule before the capsule became saturated in graphite. Because the capsules did not contain graphite, this composition is the most CO_2 -poor that could have been present in the capsule. This composition is documented in the box in the upper right of the diagram in terms of the fluid phases present and the ambient fugacities of O_2 and H_2 .

D is the bulk distribution coefficient between CO_2 and the solid assemblage). Alternatively, we can assume that the unrecovered Nd was in the solid product and that the CO_2 contained <500 pg Nd (Table 1). This suggests that $D^{\text{CO}_2/\text{solid}}(\text{Nd}) < 0.005$ for our runs (<0.0005 for runs with highest Nd contents). The $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ can be no lower than $D^{\text{CO}_2/\text{solid}}(\text{Nd})$; an upper limit on the value of K_D may be estimated if Nd contents of

TABLE 1 Experimental results

<i>P</i> (kbar)	<i>T</i> (°C)	Time (h)	Mass solid* (mg)	Mass $\text{CO}_2^\dagger$ (mg)	Mass Nd initial‡ (ng)	Mass Nd final§ (ng)	Recovery (%)	Mass Nd fluid¶ (ng)
Diopside- CO_2								
10	1,000	24	11.5	10.5	1,130 $\pm$ 23	1,100 $\pm$ 22	97.3	<0.5
10	1,000	24	10.3	9.0	515 $\pm$ 10	498 $\pm$ 10	96.7	<0.5
10	1,000	24	10.8	11.1	109 $\pm$ 2	103 $\pm$ 2	94.5	<0.5
10	1,200	24	11.2	9.2	1,100 $\pm$ 22	1,110 $\pm$ 22	100.9	<0.5
10	1,200	24	9.3	11.3	465 $\pm$ 9	472 $\pm$ 9	101.5	<0.5
10	1,200	24	10.4	10.4	105 $\pm$ 2	100 $\pm$ 2	95.2	<0.5
15	1,000	24	11.4	9.0	1,130 $\pm$ 23	1,080 $\pm$ 22	95.6	<0.5
15	1,000	24	11.2	12.1	560 $\pm$ 11	562 $\pm$ 11	100.4	<0.5
15	1,000	24	11.2	9.6	113 $\pm$ 2	109 $\pm$ 2	96.5	<0.5
15	1,200	24	9.4	10.1	920 $\pm$ 18	884 $\pm$ 18	96.1	<0.5
15	1,200	24	12.6	11.0	630 $\pm$ 13	636 $\pm$ 13	101.0	<0.5
15	1,200	24	12.0	9.7	121 $\pm$ 2	116 $\pm$ 2	95.9	<0.5
Calcite liquid- CO_2								
10	1,550	0.5	8.3	7.2	851 $\pm$ 17	858 $\pm$ 17	100.8	<0.5
10	1,550	0.5	9.6	7.6	466 $\pm$ 9	447 $\pm$ 9	95.9	<0.5
10	1,550	0.5	9.5	7.0	93 $\pm$ 2	92 $\pm$ 2	99.6	<0.5

All runs were performed in solid-media pressure vessels using the piston-out technique. The experimental techniques are given elsewhere²⁴. The analysis of materials was performed by mass-spectrometric isotope dilution using ^{145}Nd -enriched spike (see text). All analyses of the quenched CO_2 fluid were below the detection limit (~ 500 pg), as were the blanks.

* Includes both diopside glass and fine SiO_2 powder in diopside- CO_2 experiments, the ratio of diopside to silica was 2:1 in all cases; calcite in calcite liquid/ CO_2 experiments.

† Calculated from knowledge of masses of PdO and graphite added; the weight difference before and after opening capsule after quenching was within 15% relative in all cases.

‡ Determined by analysis of an aliquot of starting material. The errors were determined by replicate analyses of the standard; the within-run errors are all less than the standard replicability.

§ Determined by analysis of solid materials in inner capsule. The errors were determined by replicate analyses of the standard; the within-run errors are all less than the standard replicability.

|| Difference between masses of Nd in the initial and final solid materials as a percentage of the amount in the initial material.

¶ Determined by the analysis of acid leachate from the outer capsule.

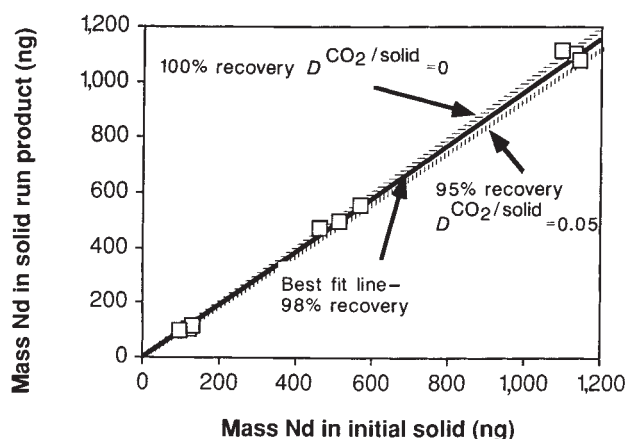


FIG. 3 Mass of Nd in diopside/silica solid run products after equilibration with CO_2 fluid as a function of Nd in the initial solid (both in nanograms); analytical errors (Table 1) are similar to the size of the symbols. The horizontally ruled line indicates equal amounts of Nd in initial and final solids. The vertically ruled line indicates 95% recovery, that is, 95% of the Nd in the initial solid was in the final solid. The solid line is the best fit to the data, indicating an average 98% recovery—within analytical error of 100% recovery—the square of the correlation coefficient being 0.998. The conclusion that the Nd is quantitatively retained in the solid is supported by the low contents of Nd in the fluid insofar as the amount of Nd in the recovered leachate from the fluid is below detection limit. The value of D is calculated for 95% recovery assuming that all 5% of the Nd not recovered resided in the fluid. This corresponds to $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ between 0.05 and 0.5. This is, however, considered to be a great overestimate of the value of K_D because the content of Nd in the fluid is believed to be <0.05 p.p.m. based on the analysis of leachates from the outer capsule (Table 1).

diopside are known. Mysen¹⁰ measured concentrations of 1.5–12 p.p.m. Sm in diopside, with higher contents occurring at higher doping levels. At our levels (15–150 ng Nd per mg diopside compared to 10 ng Nd per mg diopside in Mysen's study), a minimum content of 10 p.p.m. Nd is conservative. At that level, $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ for the runs could be no more than 0.5 (Fig. 3) using $D^{\text{CO}_2/\text{solid}}(\text{Nd}) = 0.05$. It is emphasized that this upper limit for K_D is extremely conservative. If the analysis of leachate from the outer capsule is an accurate measure of the Nd content of the fluid, values of $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ are less than 0.005.

The value of $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ can also be calculated from the calcite melt/ CO_2 experiments. The value of $K_D^{\text{CO}_2/\text{CM}}(\text{Nd})$ (where CM indicates carbonate melt) is between 0 and 0.5 (95% recovery) if it is estimated from the Nd recovered from the melts; between 0 and 0.007 if it is estimated from the leachates. $K_D^{\text{cpx}/\text{SM}}(\text{Nd})$ (where cpx indicates clinopyroxene and SM indicates silicate melt) is typically ~ 0.4 (ref. 21) and $K_D^{\text{CM}/\text{SM}}(\text{Nd})$ (ref. 12) is typically ~ 3 . Combination of these two values indicates that $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$ is no more than 0.05 if calculated from analyses of the leachate, a value comparable to that calculated above.

Natural mantle clinopyroxenes dissolve rare-earth elements by charge compensation of univalent cations in octahedral sites or of Al^{3+} in tetrahedral sites²². This allows higher rare-earth element content than those in our experiments, in which rare-earth element solution is limited by the number of vacancies in the octahedral cation sites²³. Such substitutional complexities do not apply to fluids, so Nd contents of CO_2 in experiments described here, as well as $K_D^{\text{CO}_2/\text{Di}}(\text{Nd})$, are upper bounds for natural CO_2 -rich fluids. Additional constraints on the potency of CO_2 as a metasomatic agent are provided by surface-energy studies^{19,20} that indicate that CO_2 -rich fluids do not infiltrate peridotite assemblages in the absence of external forces. We conclude that CO_2 -rich fluids are extremely poor agents of metasomatism, cryptic or otherwise, so that the search for the agent or mechanism of cryptic metasomatism should continue. □

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1. Frey, F. A. & Green, D. H. *Geochim. cosmochim. Acta* **38**, 1023–1059 (1974).
2. Wilshire, H. G. & Shervais, J. W. *Phys. Chem. Earth* **9**, 257–272 (1975).
3. Lloyd, F. E. & Bailey, D. K. *Phys. Chem. Earth* **9**, 389–416 (1975).
4. Harte, B. in *Continental Basalts and Mantle Xenoliths* (ed. Hawkesworth, C. H. & Norry, M. J.) 46–91 (Shiva, Cheshire, 1983).
5. Menzies, M. A. & Hawkesworth, C. J. *Mantle Metasomatism* (Academic, London, 1987).
6. Nixon, P. H. *Mantle Xenoliths* (Wiley, Chichester, 1987).
7. Menzies, M. A. in *Continental Basalts and Mantle Xenoliths* (ed. Hawkesworth, C. J. & Norry, M. J.) 92–110 (Shiva, Cheshire, 1983).
8. Dawson, J. B. in *Kimberlites II: The Mantle and Crust–Mantle Relationships* (ed. Kornprobst, J.) 289–294 (Elsevier, Amsterdam, 1984).
9. Menzies, M. A., Kempton, P. & Dungan, M. J. *Petrology* **26**, 663–693 (1985).
10. Mysen, B. O. *Neues Jb. Miner. Abh.* **146**, 41–65 (1983).
11. Mysen, B. O. *Am. Miner.* **64**, 274–287 (1979).
12. Wendlandt, R. F. & Harrison, W. J. *Contr. Miner. Petrol.* **69**, 409–419 (1979).
13. Egger, D. H. in *Mantle Metasomatism* (ed. Menzies, M. A. & Hawkesworth, C. J.) 21–41 (Academic, London, 1987).
14. Meen, J. K. *Geol. Soc. Am. spec. Pap.* **215**, 91–100 (1987).
15. Meen, J. K., Ayers, J. C. & Fregeau, E. J. in *Carbonatites: Origin and Evolution* (ed. Bell, K.) 464–499 (Hyman Unwin, London, 1989).
16. Wallace, M. W. & Green, D. H. *Nature* **335**, 343–346 (1988).
17. Green, D. H. & Wallace, M. W. *Nature* **336**, 459–462 (1988).
18. Boyd, F. R. & England, J. L. *J. geophys. Res.* **65**, 741–748 (1960).
19. Watson, E. B. & Brennan, J. M. *Earth planet. Sci. Lett.* **85**, 497–515 (1987).
20. Brennan, J. M. & Watson, E. B. *Earth planet. Sci. Lett.* **91**, 141–158 (1988).
21. Arth, J. G. *U. S. Geol. Surv. J. Res.* **4**, 41–47 (1976).
22. Henderson, P. in *Rare Earth Element Geochemistry* (ed. Henderson, P.) 1–32 (Elsevier, Amsterdam, 1984).
23. Harrison, W. J. & Wood, B. J. *Contr. Miner. Petrol.* **72**, 145–155 (1980).
24. Meen, J. K. *Contr. Miner. Petrol.* **97**, 33–351 (1987).
25. Crank, J. *The Mathematics of Diffusion* (Clarendon, Oxford, 1975).
26. Holloway, J. R. in *Thermodynamics in Geology* (ed. Fraser, D. G.) 301–325 (Reidel, Boston, 1977).

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A 6,000 year history of Amazonian maize cultivation

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WE present pollen and phytolith evidence for maize (*Zea mays* L.) cultivation in lowland Ecuadorian Amazonia as early as 5,300 radiocarbon years BP (before present), equivalent to about 6,000 calendar years BP¹. This date for maize cultivation is more than 2,000 years earlier than any previously reported from the Amazon basin². Although maize has been cultivated for at least 7,000 years in Mexico^{3,4}, the manner of its dispersal through South America is still uncertain^{2–6}. Evidence from coastal Ecuador⁶ suggests that maize had been taken south across the equator by 7,000 years BP. The oldest macrofossil evidence from Ecuador, however, is from about 3,400 years BP⁷. Our discovery of *Zea* microfossils in Amazonian lake sediments from Ecuador at about 6,000 years BP suggests that maize cultivation spread into the Amazon lowlands soon after its arrival in South America.

The data presented here come from sediments of Lake Ayauchi, a 0.04 km², oligotrophic lake occupying a steep-sided basin of apparently volcanic origin at ~ 500 -m elevation. The lake is situated ($2^{\circ}5'5$, $78^{\circ}1'W$) at the foot of the Andes, in the southeastern part of Ecuadorian Amazonia. Lowland tropical *terra firma* rainforest covers the crater rim, growing close to the edge of the water. Parallel sediment cores were raised from beneath 20 m of water with a modified Livingstone piston sampler and returned to the Columbus laboratory in the original core tubes, where they were X-rayed before opening. Horizontal banding patterns demonstrated that the sediments were not disturbed during coring, and four internally consistent radiocarbon dates show that the deposits span the last 7,000 radiocarbon years. Lake morphometry, core stratigraphy, dating and pollen analysis are described elsewhere⁸.

Subsamples were taken from the longest core (3.26 m) for palynological and phytolith analysis. Standard pollen extracts were made from measured volumes of sediment and mounted in glycerol^{9,10}. Quantitative pollen counts were based on 200 non-*Cecropia* pollen; thereafter further slides were searched for rare pollen types, such as Bombacaceae and *Zea*, although no count was made. This procedure of searching preparations of many thousands of pollen grains to identify the presence of taxa that contribute few but distinctive pollen to sediment basins can provide strong evidence for the local presence of taxa, like *Zea*, whose pollen disperses poorly¹¹. All Gramineae pollen grains were measured and *Z. mays* pollen was identified on the basis of size ($>95\text{ }\mu\text{m}$; most were in the range $100\text{--}135\text{ }\mu\text{m}$) and its characteristic surface pattern under phase-contrast illumination. Phytoliths were isolated by density gradients and concentrations were determined by the aliquot method¹².

The pollen history shows that the forests that grew around Ayauch¹ between 7,100 and 5,300 years BP were rich in taxa which could have belonged within either a mature or an immature forest, for example *Alchornea*, Anacardiaceae, *Didymopanax*, *Ficus* and *Urticaceae/Moraceae*⁸. Evidence that this was probably a mature forest comes from the presence of members of the Bombacaceae: *Pachira aquatica*, *Pseudobombax* and *Ochroma*, and low values for the disturbance indicator *Cecropia* (Fig. 1). The Bombacaceae are entomophilous and so even low

numbers of these pollen types probably indicate a substantial bombacaceous canopy component.

The first occurrence of *Z. mays* pollen and phytoliths is at 2.4 m in sediments bounded by uncorrected radiocarbon dates of $4,570 \pm 70$ years BP (Beta 20956) and $7,010 \pm 130$ years BP (I-13, 306), yielding an interpreted age of 5,300 BP. Applying the Pearson *et al.* calibration¹ gives a calendar age of $\sim 5,970\text{--}6,190$ years BP. The first evidence for *Zea* is associated with a rise in *Cecropia* and with the disappearance of the Bombacaceae from the pollen record (*Pseudobombax* and *Pachira aquatica* are last recorded at 2.34 m; *Ochroma* is recorded twice more at a much higher level in the core). The *Z. mays* pollen at 2.40 m measured $102\text{ }\mu\text{m}$ (Fig. 2) in samples in which no other Gramineae pollen type exceeded $45\text{ }\mu\text{m}$. The appearance of *Zea* coincides with a rise in the pollen representation of herb taxa characteristic of disturbed ground, including three types of *Acalypha*, Chenopodiaceae/*Amaranthus*, Caryophyllaceae, Gramineae, Liguliflorae, *Plantago* and Tubuliflorae. In overlying samples there was a sporadic occurrence of *Zea* pollen between 2.4 m and 0.5 m, consistent with shifting agriculture resulting in periodic site abandonment. *Zea* pollen comprise 0.5 to 1% of non-*Cecropia* pollen in some samples, and *Zea* phytoliths increase dramatically in abundance at about 2,500 radiocarbon years BP, perhaps representing an intensification of cultivation in and around the basin of Ayauch¹. Sediment

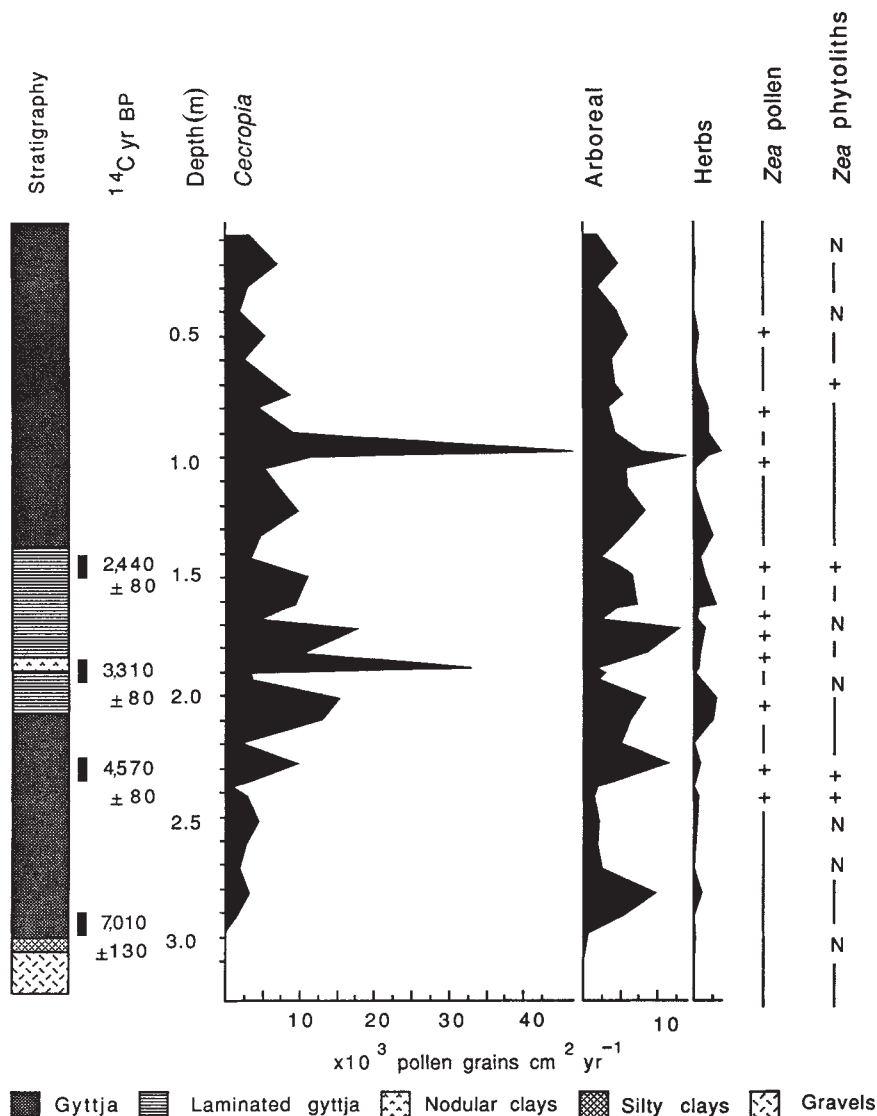


FIG. 1 Sediment stratigraphy, pollen influx values and representation of maize phytoliths from Lake Ayauch¹, Ecuador. 'Arboreal taxa' includes: *Alchornea*, Bombacaceae, Moraceae, Tiliaceae, Ulmaceae, Urticaceae; 'Herb taxa' includes: *Acalypha*, *Alternanthera*, Cyperaceae, Gramineae, Malvaceae. 'N' denotes level searched for *Zea* phytoliths and none found. Laboratory numbers of ¹⁴C dates are, from top to bottom: Beta 20954–20956, and I-13,306.

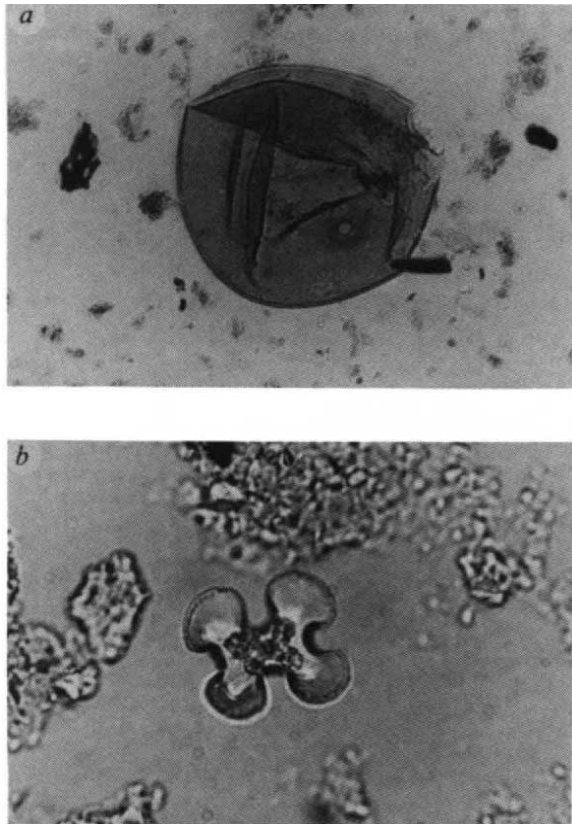


FIG. 2 Fossil *Zea mays* from 2.4 m, about 5300 uncorrected years BP. a, pollen 102 μm in length; b, phytolith 23 μm $\times$ 20 μm .

stratigraphy suggests that maize may have been cultivated within the basin at times when the water level of the lake was lower than at present. The final abandonment of agriculture at this site at $\sim$ 800 radiocarbon years BP may have been due to the rising lake levels, which were a consequence of Amazonian late-Holocene climatic change⁸. The disappearance of *Zea* from the pollen and phytolith records at 0.5 m coincides with a decrease in herb representation, suggesting the loss of open habitats.

The occurrence of maize in the Amazon basin as early as 6,000 years BP is consistent with its presence in the contemporary pre-ceramic cultures of lowland coastal Ecuador^{6,13,14}, and establishes an early agricultural tradition in the basin itself. Furthermore, the 'early' arrival of maize in Amazonia is consistent with hypotheses of early dispersals of maize from Mexico to South America^{5,6}. Roosevelt has argued that mid-Holocene Amazonian human populations were protein-limited and correlates population expansion with a 'late' introduction of maize². The new data from Ayauch¹ suggest, however, that the technology of maize cultivation swept rapidly into South America, and that there was not a significant time-lag before the new technology was adopted in Amazonia. The apparent delay between maize introduction and human population increase may be artefactual, in that archaeological sites are few in this landscape, which is continuously perturbed by rivers^{15,16}, or human population increase may be related to the introduction of higher-yielding varieties of maize; this argument has been advanced to account for the time-lag between onset of agriculture and year-round village occupation in Mexico¹⁷. Another implication of these data is that historical *terra firma* Amazonian forests were in part accommodated to several thousand years of human exploitation. Similar disturbance by humans in Central America has been cited as a form of intermediate disturbance promoting high species diversity in *terra firma* forests¹⁸. Our

data suggest that this argument can be extended to the *terra firma* forest of Amazonia. □

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1. Pearson, G. W., Pilcher, J. R., Baillie, M. G., Corbett, D. M. & Qua, F. *Radiocarbon* **28**, 911–934 (1986).
2. Roosevelt, A. C. *Parmana: Prehistoric Maize and Manioc Subsistence Along the Amazon and Orinoco* (Academic, New York, 1980).
3. MacNeish, R. S. in *The Prehistory of the Tehuacan Valley* (ed. Beyers D.) Vol. 1, 290–309 (University of Texas Press, Austin, 1967).
4. Beadle, G. W. in *The Origins of Agriculture* (ed. Reed, C. A.) 615–635 (Mouton, The Hague, 1977).
5. Lathrap, D. W. *Ancient Ecuador: Culture, Clay and Creativity 3000–300 B.C.* (Field Museum of Natural History, Chicago, 1975).
6. Pearsall, D. M. & D. R. Piperno *Am. Antiq.*, in the press.
7. Lippi, R. N., Bird, R. M., & Stemper, D. M. *Am. Antiq.* **49**, 118–124 (1984).
8. Bush, M. B. & Colinvaux, P. A. *Vegetatio* **76**, 141–154 (1988).
9. Faegri, K. & Iversen, J. *A Textbook of Pollen Analysis* (Blackwell Scientific, Oxford, 1975).
10. Stockmarr, J. *Pollen Spores* **13**, 615–621, (1971).
11. Jones, W. D. & Newell, L. C. *Univ. Nebraska Agric. exp. stat. Res. Bull.* **148** (1946).
12. Piperno, D. R. *Phytolith Analysis: An Archaeological and Geological Perspective* (Academic, San Diego, 1988).
13. Pearsall, D. M. *Science* **199**, 177–178 (1978).
14. Zevallos, M. C. et al. *Science* **196**, 385–389 (1977).
15. Colinvaux, P. A., Miller, M. C., Liu, K.-b., Steinitz-Kannan, M. & Frost, I. *Nature* **313** 42–45 (1985).
16. Salo, J. et al. *Nature* **322**, 254–258 (1986).
17. Fiedel, S. J. *Prehistory of the Americas* (Cambridge University Press, 1987).
18. Hubbel, S. P. *Science* **203**, 1299–1309 (1979).

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Modulation of the mechanical properties of spider silk by coating with water

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THE orb web of the garden cross spider *Araneus diadematus* is made up of two types of thread with distinctive mechanical properties: stiff radial threads which sag when contracted by only 10%, and elastic circumferential 'capture' threads which can be contracted to less than 5% of their original length in the web without sagging^{1–5}. Everyday silk is stiff, but can be plasticized by water^{6–9}. It is known that capture threads are coated with a thin layer of aqueous glue^{10–12}, and the question arises as to whether this layer can account for their remarkable elasticity. The evidence presented here suggests that the differences between radial and capture threads are primarily the result of the water coating of the capture threads, which both plasticizes the silk and provides additional elasticity from the surface tension of the liquid.

The capture thread consists of a pair of core fibres evenly coated on extrusion with a viscous liquid¹³ consisting of 80% water and containing amino acids, lipids and salts^{14–16}. Because a liquid cylinder of this kind, supported along its axis, is unstable¹⁷ it spontaneously flows into regularly spaced droplets strung along the core fibres (Fig. 1a). The fact that the ionic solution conducts electricity, in contrast to the core fibres, allowed us to study the coat by measuring the resistance of the necked liquid cylinders that connect the droplets. Our data (not shown) demonstrated that the resistance, typically 2×10^{10} ohm for a 10-mm long capture thread from a 200-mg *Araneus*, increases in a reproducible manner as the thread is stretched, and is approximately proportional to the square of the length. This behaviour is indicative of an elastic conducting cylinder of constant volume. After drying the thread in air dehydrated by phosphorus pentoxide (a reversible process), its resistance was $\sim 10^{13}$ ohm; the same thread when washed in deionized water and dried behaved like naturally dry silk, having a resistance larger than the range of our instrument (2×10^{14} ohm; Wide Range Megohmmeter 11801, Pye and Co.). This test allowed us to confirm the presence or absence of a continuous water coat.

We found that old *Araneus* occasionally exhaust their supply of coating liquid yet continue to lay down spiral capture threads consisting only of core fibres. This allowed us to compare directly, and without a potentially harmful wash, the mechanics of wet and dry core fibres. Unlike its wet counterpart, uncoated spiral thread whose dryness was confirmed by resistance measurement was loose even *in situ*, suggesting that, when laid down, the coated thread is not pre-tensed. (The animal's building behaviour remains the same whether coated or uncoated thread is paid out.) Moreover, such dry threads showed only inelastic recoil when stretched, in sharp contrast to their wet counterparts (see Fig. 2a). It seemed therefore that the taughtness of the capture threads is due to the coating and not to any pre-tension of the actual core fibres. Further experiments on wet and dry spiral capture threads as well as radial threads led us to divide the elastic characteristics of coated spider silk into three parts according to its response to: extreme contraction, extreme extension and intermediate extension/contraction.

Examination by microscope of a normal capture thread, when contracted to less than half its web-length, revealed that, although the core fibres slacken, overall taughtness is

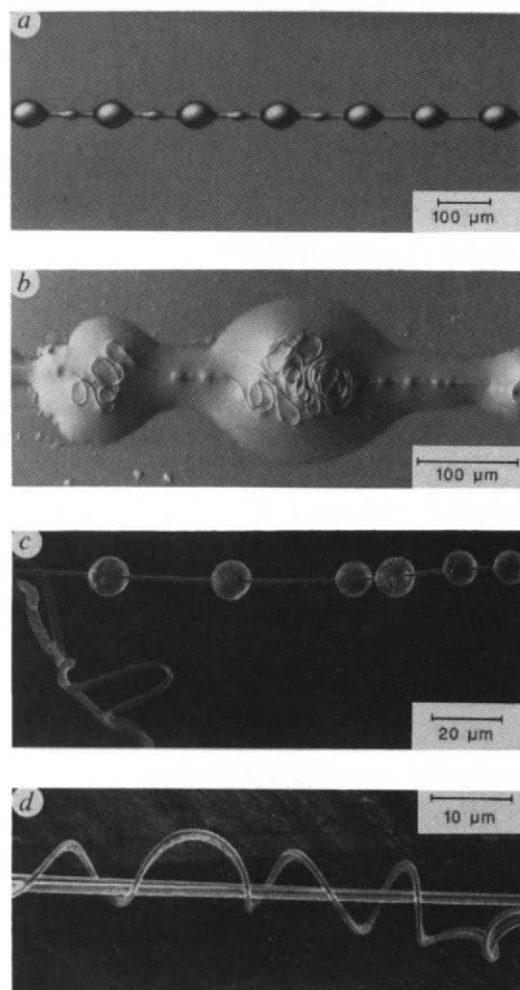


FIG. 1 a, View of an unstretched capture thread suspended in air. b, View of a similar thread contracted to 50% web-length and then laid onto a glass slide to show the loose core fibres gathered within the larger coalesced glue droplets. The nodules distributed along the core fibres may be related to the original droplet distribution but as yet we have no evidence for this. c, Scanning electron microscope view of a capture thread and a radial. The droplets of the viscid coat have been preserved using an osmium steaming method (developed by Dr Barry Juniper, Department of Plant Sciences, Oxford). d, Scanning electron microscope view of a tight radial thread encircled by a capture thread which lacks the glue.

maintained, presumably by the surface tension of the coat. The glue droplets begin to merge and the larger droplets act like tiny windlasses, inside which balls of loose core fibre collect (Fig. 1b). When the thread is stretched the core fibres unwind again. Although sufficient to maintain taughtness at large contractions, the coat's surface tension creates a force very much smaller than that measured for lesser contractions and small extensions (see Fig. 2b). Assuming the surface tension of water to act around a cylinder of diameter $1\text{ }\mu\text{m}$, this force would be about $2.3 \times 10^{-7}\text{ N}$. At intermediate contraction, the plasticizing effect of the absorbed water⁷⁻⁹ led to reversible elasticity.

Figure 2 shows that for wet capture thread and intermediate extensions of up to $\sim 200\%$ web-length, the resisting force is small and reversible, whereas at larger extensions this force rises dramatically and shows some hysteresis. Dry capture thread on

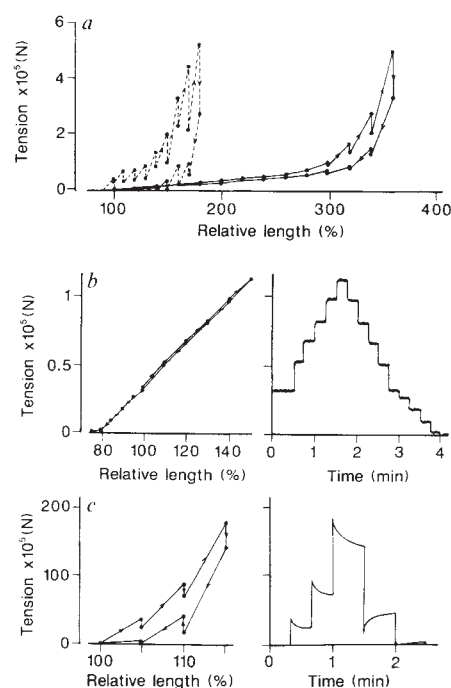


FIG. 2 Comparison between the static and dynamic behaviour of naturally wet and dry spider silk. In each case the imposed change in length was rapid (1 s) and the system was then allowed to relax before the next change. a, Comparison between the tension versus extension characteristics of coated (full line) and uncoated (broken line) spiral capture threads (length 5 mm, diameter of single fibre, $0.7\text{ }\mu\text{m}$) from the same web of *Araneus diadematus*. b, Characteristic of the coated thread (length 10 mm; diameter, $\sim 1\text{ }\mu\text{m}$) in greater detail showing its reversibility and linearity for intermediate extensions (up to 150% web-length). At contractions below 50% web-length the tension is small but sufficient to prevent sag. This force is a result largely of the surface tension of the coating liquid. On the right is the tracing of the chart recorder showing the tension as a function of time from which the characteristic on the left was drawn. c, The characteristic for dry radial thread (length 10 mm, diameter of single fibre ($\sim 1.4\text{ }\mu\text{m}$) from the same web with the accompanying recorder trace. The relatively inelastic behaviour closely resembles that of the dry spiral thread shown in Fig. 2a, the differences in force we attribute to the thicker thread.

METHODS. A microbalance made by C.I Electronics Ltd. was adapted for our purposes. The balanced position is sensed optically and an out-of-balance signal is amplified and fed back to torque coils on the balance so as to maintain the arm position. A chart recorder continuously records a voltage proportional to the current to the torque coils and hence to the balancing force. The balance, which has a response time much less than a second, was calibrated with known weights. The spider threads were mounted vertically between the balance arm above and a calibrated micro-manipulator below, so that the lower end of the thread could be raised or lowered smoothly and quickly (1 s) by known distances. Both attachment hooks were coated with clear nail varnish just before the threads were placed to anchor the ends securely.

the other hand shows much greater stiffness and hysteresis even at low extensions, and resembles a dry radial thread (Fig. 2c), albeit with a smaller force, presumably because of its smaller diameter. The distinction between the kinetic behaviour of dry and coated threads is best seen in the time dependence of the tensile characteristics of the thread following a small but rapid (1 s) change in its length (Figs 2b, c). The coated thread immediately assumes the new equilibrium value whereas the dry thread on extension exhibits an initially large force which decays in time.

To demonstrate that it is the water and not the other chemicals present in the coat which determines the changes in thread characteristic, we determined the time characteristic of the force in a radial thread first in air (dry) and then under water (wet). Not only did immersion in water lower the tension for a given extension (as reported in refs 7 and 9), but it ensured the rapid attainment of the new equilibrium (Fig. 3a); a radial thread under water behaves therefore like a coated capture thread in air. Conversely, removal of just the water from a coated thread gave rise to large tensions for small extensions, and a dramatic loss of reversibility (Fig. 3b). In ambient air, however, the original characteristics of the thread were immediately restored, including its rapidly reversible elasticity (Fig. 3b).

The incorporation of the mechanical properties of the two types of silk (elastic and inelastic) into one web has obvious advantages. The tightly strung and stiff radial threads provide support for spider and prey², and transmit informative vibrations¹⁸. On the other hand, the ability to maintain tension when hugely stretched and relaxed in rapid succession is essential to the coated capture threads, because it prevents single strands from touching and agglutinating when the web is buffeted and distorted by the wind. Moreover, it is important for the capture of prey that the capture threads can absorb the high kinetic

energy of the insect's impact, and then entangle without offering any purchase which might aid a struggle to escape^{1,3,4}. As the silk of most spiders is dry¹¹, and as all but one of the eight types of silk produced by *Araneus* are dry¹⁹, it is reasonable to assume that the dry state is ancestral for spider silk. The inherent weakness of dry silk (that it loses tension when wet) is thus turned into an advantage by the araneid spiders by their evolving a type of silk gland that produces the liquid for coating the dry silk. The resulting combination of the various silks into a single network, with a pivotal role for the converted (wet) silk, provides the araneid orb web with its unique properties and turns it into a very successful trap¹⁻³. □

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1. Denny, M. W. *J. exp. Biol.* **65**, 485-506 (1976).
2. Witt, P. N., Reed, C. & Peakall, D. B. *A Spider's Web* (Springer, Heidelberg, 1968).
3. Gosline, J. M., DuMont, M. E. & Denny, M. W. *Endeavour* **10**, 37-43 (1986).
4. Craig, C. L. *Biol. J. Linn. Soc. Lond.* **30**, 135-163 (1987).
5. DeWilde, J. *Arch. neerl. Physiol.* **28**, 118-131 (1943).
6. Magoshi, J., Misuide, M. & Magoshi, Y. *J. polymer Sci.* **17**, 515-520 (1979).
7. Work, R. W. *Text. Res. J.* **47**, 650-662 (1977).
8. Gosline, J. M., Denny, M. W. & DuMont, M. E. *Nature* **309**, 551-552 (1984).
9. Work, R. W. *J. exp. Biol.* **118**, 379-404 (1985).
10. Foelix, R. F. *Biology of Spiders* (Harvard University Press, 1984).
11. Tillinghast, E. K. & Townley, M. in *Ecophysiology of Spiders* (ed. Nentwig, W.) 203-210 (Springer, Heidelberg, 1987).
12. Eisner, T., Alsop, R. & Ettershank, G. *Science* **146**, 1058-1061 (1964).
13. Peters, J. M. in *Ecophysiology of Spiders* (ed. Nentwig, W.) 187-202 (Springer, Heidelberg, 1987).
14. Fischer, F. G., Brander, J. & Hoppe-Seylers, Z. *Physiol. Chem.* **320**, 92-102 (1960).
15. Schildknecht, H., Kunzelmann, P. & Kuhn, C. *Naturwissenschaften* **29**, 98-99 (1960).
16. Anderson, C. M. & Tillinghast, E. K. *Physiol. Entomol.* **5**, 101-106 (1980).
17. Rayleigh (Lord), *Phil. Mag.* **34**, 145-154 (1892).
18. Masters, W. M. & Markl, H. *Science* **213**, 363-365 (1981).
19. Kovoor, J. in *Ecophysiology of Spiders* (ed. Nentwig, W.) 160-186 (Springer, Heidelberg, 1987).

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Fibronectin inhibits the terminal differentiation of human keratinocytes

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IN the epidermis proliferation of keratinocytes is restricted to the basal layer, which is in contact with the basement membrane, and cells undergo terminal differentiation as they move upwards through the suprabasal layers. In stratified cultures of human keratinocytes, upward migration is a consequence, not a cause, of terminal differentiation¹ and occurs because keratinocytes become less adhesive to their substratum and to one another². Most keratinocytes can be induced to differentiate to completion by placing them in suspension in methylcellulose³: within 12 h DNA synthesis is irreversibly inhibited and by 24 h most cells express involucrin (ref 4; P. A. Hall, J.C.A. and F.M.W., unpublished observations). Here we report that when fibronectin is added to the methylcellulose, keratinocytes still withdraw from the cell cycle, but induction of involucrin expression is largely inhibited. The effect of fibronectin is concentration- and time-dependent and is mediated by a receptor of the integrin family⁵. These results provide an explanation for why overt terminal differentiation is normally restricted to suprabasal cells, whereas cell-cycle withdrawal occurs within the basal layer; they also have important implications for the mechanism of epidermal wound healing. Furthermore, our data show that the binding of an extracellular matrix protein to its receptor can regulate differentiated gene expression in the absence of changes in cell shape.

Purified extracellular matrix proteins to which keratinocytes adhere⁶ were mixed with methylcellulose at the time of plating

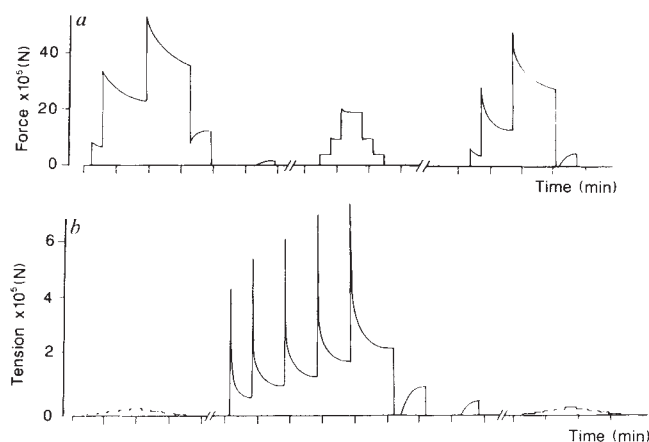


FIG. 3 Comparison of the dynamic behaviour of spider silk with experimentally altered wet and dry states. *a*, Time-dependence of the recorded force when a radial thread (length, 6 mm) is cycled through the same series of three extensions and three contractions. In the first trace the thread is in air, in the second it is immersed in de-ionized water containing 0.9% Teepol and in the third it is returned to air. *b*, Time-dependence of the extension recorded for a coated capture spiral thread (length 10 mm) which is cycled through five equal extensions from 100% web-length to 150% web-length and back. The first trace is recorded in the laboratory atmosphere of 50% relative humidity and 19 °C, the second shortly after in air dried by P₂O₅, and the third after ambient air had been re-admitted.

METHODS. For *b*, the balance was used as described in the legend to Fig. 2; for *a*, the thread was clamped horizontally between Perspex jaws 6 mm apart. In the latter case, the hook from the balance applied a vertical upward force at the centre of the clamped thread (hence the different ordinate units in *a* and *b*). The cycle in *a* corresponds to positions of the hook displayed first downward then upward by 0.5, 1.0, 1.5 mm from the position in which the thread was straight; the cycle in *b* corresponds to displacements by 1.0, 1.2, 1.3, 1.4 and 1.5 mm. The Teepol was added to the water to lower the surface tension forces acting on the balance wire where it entered the water bath.

the cells. The starting population contained 25–30% involucrin-positive cells. After 24 h in suspension in methylcellulose alone, or with added bovine serum albumin (BSA) or gelatin (both non-adhesive proteins), this increased to ~80% (Table 1). Neither collagen types I and IV, nor laminin had any effect on terminal differentiation, but fibronectin caused a significant reduction in the number of involucrin-positive cells ($P=0.001$) (Table 1). Because fibronectin preparations can contain bound transforming growth factor- β (TGF- β)⁷ and TGF- β can promote anchorage independent growth of cells⁸, we tested whether TGF- β alone had any effect on terminal differentiation. No effect was found (Table 1).

The effect of fibronectin is dose-dependent, with maximal inhibition at $\geq 75 \mu\text{g ml}^{-1}$ (0.19 μM) (Fig. 1a). Even at the highest concentration, the number of involucrin-positive cells is greater than in control cultures (Table 1). Thus within the starting population of involucrin-negative cells, ~30% are not susceptible to fibronectin, possibly because they are already committed to undergo terminal differentiation.

The time course of suspension-induced involucrin expression was examined in the presence of fibronectin or BSA (Fig. 1b). In BSA-treated cultures the percentage of involucrin-positive cells began to rise after 6 h and increased rapidly between 6 h and 10 h, after which the rise was more gradual. In fibronectin-treated cultures, the proportion of involucrin-positive cells reached a maximum of 45%.

The length of time for which keratinocytes remained sensitive to fibronectin was measured by delaying the addition of fibronectin for up to 8 h after plating the cells (Fig. 1c). For maximum inhibition of terminal differentiation it was necessary to add fibronectin within 2 h; by 4 h, addition of fibronectin had little effect, even though the percentage of cells expressing involucrin did not start to rise until 6 h in control cultures (Fig. 1b).

In the epidermis, irreversible withdrawal from the cell cycle occurs in the basal layer and precedes overt terminal differentiation⁹. We measured [³H]thymidine incorporation in control

and fibronectin-treated cells that had been in suspension for 2 h or 20 h. As shown in Fig. 2, there is no difference between control and treated cells; thus fibronectin does not prevent cell-cycle withdrawal.

The main cell-binding site of fibronectin is a short peptide, RGDS¹⁰, which is recognized by members of the integrin superfamily of receptors^{5,11}. Antibodies to the $\alpha_5\beta_1$ fibronectin receptor inhibit keratinocyte attachment to fibronectin (J. C. A. and

TABLE 1 Effect of extracellular matrix proteins and TGF- β on suspension-induced terminal differentiation

Sample	Involucrin-positive cells (%) $\pm$ s.e.m.
Starting population	24.8 $\pm$ 1.02
After 24 h suspension:	
no treatment	79.0 $\pm$ 0.82
+fibronectin	51.1 $\pm$ 0.89
+collagen I	78.0 $\pm$ 2.53
+collagen IV	77.0 $\pm$ 1.81
+laminin	73.6 $\pm$ 2.77
+BSA	78.0 $\pm$ 1.82
+gelatin	76.3 $\pm$ 2.92
+TGF- β	75.3 $\pm$ 1.67

Stock cultures of newborn human foreskin keratinocytes (strains a, c and t; passage numbers 3–10) were grown as described previously²¹. Pre-confluent cultures were disaggregated in trypsin/EDTA and resuspended at a concentration of 10^5 cells ml^{-1} in medium supplemented with 1.45% methylcellulose (4,000 centipoises, Aldrich). Aliquots (2 ml) were plated in 35-mm-diameter bacteriological plastic dishes coated with 0.4% polyHEMA; this ensured that there was no cell–substratum adhesion, even in the presence of fibronectin. After incubation for 24 h at 37 °C the methylcellulose was diluted with PBS and the cells recovered by centrifugation. The cells were fixed and stained for involucrin as described previously²², using a monoclonal antibody kindly provided by T. Rupniak²³. TGF- β was added to give a final concentration of 20 ng ml^{-1} ; all other proteins were used at 100 $\mu\text{g ml}^{-1}$. Human plasma fibronectin was purchased from Calbiochem or Biogenesis; human placental collagen type IV, mouse EHS laminin and BSA from Sigma; bovine collagen type I (Vitrogen 100) from Collagen Corporation; gelatin from BDH and human placental TGF- β from R and D Systems. Duplicate dishes were used for each experiment and at least 500 cells were scored per dish. Values shown are means of replicate experiments $\pm$ s.e.m. In *t*-test calculations, treated samples were compared with the untreated sample after 24 h in suspension.

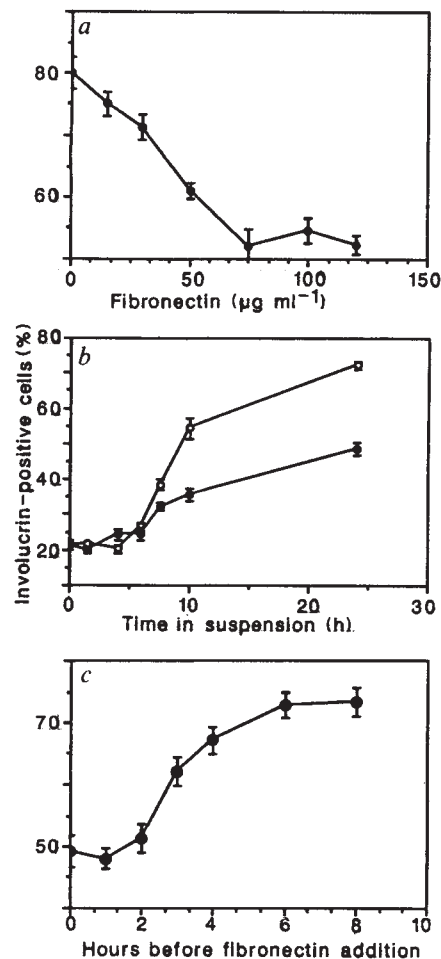


FIG. 1 Kinetics of fibronectin inhibition of terminal differentiation. *a*, Different concentrations of fibronectin were added at the time of suspending cells and the percentage of involucrin-positive cells measured 24 h later. *b*, Percentage of cells expressing involucrin was measured after different times in suspension in the presence of BSA (75 $\mu\text{g ml}^{-1}$) (○) or fibronectin (●). *c*, Fibronectin (75 $\mu\text{g ml}^{-1}$) was added at different times after plating and the cells were collected after 24 h in suspension. Data points are the mean $\pm$ s.e.m. of triplicate (*a*, *b*) or duplicate (*c*) experiments.

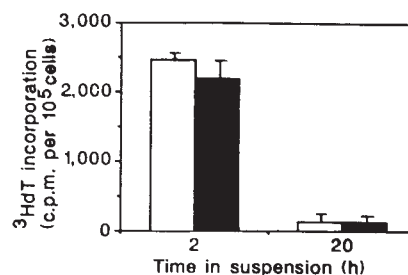


FIG. 2 Incorporation of [³H]thymidine in methylcellulose alone (□) or +fibronectin (100 $\mu\text{g ml}^{-1}$) (■) after 2 or 20 h in suspension. Cells were labelled with [³H]thymidine (20 $\mu\text{Ci ml}^{-1}$) (Amersham, specific activity 25 Ci per mmol⁻¹) for 1 h before harvesting and transferred to GF/C filters (Whatman). Incorporated counts were measured after precipitation with trichloroacetic acid.

F. M. W., unpublished observation), suggesting that keratinocytes bind fibronectin by means of this integrin. The gelatin- and heparin-binding fragments of fibronectin do not inhibit terminal differentiation, but the cell-binding fragment is as effective at inhibiting as the intact molecule (Table 2). GRGDSP has a small inhibitory effect, which may reflect the relatively low affinity of this peptide for the receptor¹². Anti-fibronectin receptor IgG was slightly more inhibitory than intact fibronectin, as only 42% of the cells were involucrin-positive after 24 h. Fab fragments were as effective as whole IgG at inhibiting, suggesting that ligand-mediated receptor clustering is not required for inhibition of terminal differentiation.

In conclusion, we have shown that fibronectin inhibits the onset of involucrin synthesis in cultured keratinocytes, an event that normally occurs in suprabasal cells, without preventing cell-cycle withdrawal, which occurs in the basal layer. To have an inhibitory effect fibronectin must be added at least 4 h before the time when involucrin expression would normally begin to increase (Fig. 1b). We therefore believe that fibronectin is acting on commitment events before overt terminal differentiation and that these are distinct from the events that result in cell-cycle withdrawal.

It is interesting that fibronectin, which is present in relatively small amounts in the epidermal basement membrane¹³, inhibits terminal differentiation. The most likely explanation is that in cultured keratinocytes the fibronectin receptor is up-regulated¹⁴. Our results may have physiological relevance to wound healing, because the fibronectin receptor is also activated in keratinocytes at the edge of wounds¹⁴ and fibronectin is a major component of the provisional matrix over which keratinocytes migrate¹⁵.

Positive or negative effects of fibronectin on differentiation have been reported in other *in vitro* models¹⁶⁻²⁰. In each case, however, addition of fibronectin caused changes in cell shape and so it was impossible to distinguish a true inductive stimulus from an indirect effect. In keratinocytes, involucrin expression

can be regulated by cell-substratum contact area and hence by cell shape⁴, but the present experiments demonstrate that fibronectin can regulate differentiated gene expression through a receptor of the integrin family, without changes in cell shape or cell-cell contact. These observations are consistent with the idea that the proportion of extracellular matrix receptors that are occupied regulates overt terminal differentiation. Finally, the experimental model described here enables the signals that regulate terminal differentiation to be separated from those controlling proliferation. □

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1. Watt, F. M. & Green, H. *Nature* **295**, 434-436 (1982).
2. Watt, F. M. *J. Cell Biol.* **98**, 16-21 (1984).
3. Green, H. *Cell* **11**, 405-416 (1977).
4. Watt, F. M., Jordan, P. W. & O'Neill, C. H. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5576-5580 (1988).
5. Ruoslahti, E. *A. Rev. Biochem.* **57**, 375-413 (1988).
6. Watt, F. M. *J. Cell Sci. Suppl.* **8**, 313-326 (1987).
7. Fava, R. A. & McClure, D. B. *J. cell Physiol.* **131**, 184-189 (1983).
8. Anzano, M. A., Roberts, A. B., Smith, J. M., Sporn, M. B. & De Larco, J. E. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6264-6268 (1983).
9. Potten, C. S. & Morris, R. J. *J. Cell Sci. Suppl.* **10**, 45-62 (1988).
10. Pierschbacher, M. D. & Ruoslahti, E. *Nature* **309**, 30-33 (1984).
11. Hynes, R. O. *Cell* **48**, 549-554 (1987).
12. Akiyama, S. K. & Yamada, K. M. *J. Biol. Chem.* **260**, 10402-10405 (1985).
13. Stenman, S. & Vaheri, A. *J. exp. Med.* **147**, 1054-1064 (1978).
14. Grinnell, F., Toda, K.-I. & Takashima, A. *J. Cell Sci. Suppl.* **8**, 199-209 (1987).
15. Colvin, R. B. in *Fibronectin* (ed. Mosher, D. F.) 213-252 (Academic, New York, 1989).
16. Pennypacker, J. P., Hassell, J. R., Yamada, K. M. & Pratt, R. M. *Exp. Cell Res.* **121**, 411-415 (1979).
17. Podleski, T. R., Greenberg, I., Schlessinger, J. & Yamada, K. M. *Exp. Cell Res.* **122**, 317-326 (1979).
18. West, C. M. *et al. Cell* **17**, 491-501 (1979).
19. Spiegelman, B. M. & Ginty, C. A. *Cell* **35**, 657-666 (1983).
20. Patel, V. P. & Lodish, H. F. *J. Cell Biol.* **105**, 3105-3118 (1987).
21. Adams, J. C. & Watt, F. M. *J. Cell Biol.* **107**, 1927-1938 (1988).
22. Read, J. & Watt, F. M. *J. invest. Dermat.* **90**, 739-743 (1988).
23. Rupniak, H. T., Turner, D. M., Wood, E. J. & Cunliffe, W. J. *J. invest. Dermat.* **87**, 164 (1986).
24. Zardi, L. *et al. Eur. J. Biochem.* **146**, 571-579 (1985).
25. Yamada, K. M. In *Fibronectin* (ed. Mosher, D. F.) 47-121 (Academic, New York, 1989).
26. Dedhar, S., Argaves, W. R., Suzuki, S., Ruoslahti, E. & Pierschbacher, M. D. *J. Cell Biol.* **105**, 1175-1182 (1987).

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TABLE 2 Effect of fibronectin fragments, peptides and fibronectin-receptor antibodies on suspension-induced terminal differentiation

Sample	Involucrin-positive cells (%) ±s.e.m.
Starting population	26.1 ± 1.0
After 24 h suspension	
+BSA	79.3 ± 0.3
+intact fibronectin	52.1 ± 1.4
+cell-binding fragment	55.9 ± 1.2
+gelatin-binding fragment	78.0 ± 2.7
+heparin-binding fragment	77.0 ± 2.9
+GRGDSP peptide	61.2 ± 2.4
+GRGESP peptide	75.0 ± 2.6
+anti-FN-R IgG	41.9 ± 2.4
+anti-FN-R Fab	43.2 ± 1.3
+Non-immune IgG	75.3 ± 2.4

The *M*_{40,000} heparin-binding and the *M*_{45,000} gelatin-binding α -chymotryptic fragments of fibronectin were obtained from Biogenesis. The *M*_{110,000} cell-binding thermolysin fragment²⁴ was a gift from Martin Humphries and had about the same activity as intact fibronectin in cell-adhesion assays; peptides from the IIIC region²⁵ were inactive (results not shown). All fragments were used at concentrations equimolar to 75 μ g ml⁻¹ intact fibronectin. GRGDSP and GRGESP peptides were purchased from Peninsula Laboratories and used at a final concentration of 5 mM, which, in the case of GRGDSP, caused maximal inhibition of keratinocyte adhesion to fibronectin; GRGESP was inactive. The anti-fibronectin receptor IgG (anti-FN-R IgG) was obtained by immunizing a rabbit with intact basal keratinocytes; by the criterion of one-dimensional peptide mapping of immunoprecipitates of surface-iodinated keratinocytes, this antiserum recognizes the same α - and β -chains as an antiserum raised against $\alpha_5\beta_1$, kindly provided by E. Ruoslahti²⁶. Anti-FN-R IgG was used at the optimal concentration (0.5 mg ml⁻¹) for inhibiting keratinocyte adhesion to fibronectin, and non-immune rabbit IgG (Sigma) was used at the same concentration. IgG, Fab and peptides were not toxic to keratinocytes.

Human $\gamma\delta^+$ T cells respond to mycobacterial heat-shock protein

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MOST T cells recognize antigen through the T-cell antigen receptor (TCR) $\alpha\beta$ -CD3 complex on the T-cell surface¹. A small percentage of T cells, however, do not express $\alpha\beta$ but a second type of TCR complex designated $\gamma\delta$ (ref. 2). Unlike $\alpha\beta^+$ lymphocytes, $\gamma\delta^+$ lymphocytes do not generally express CD4 or CD8 molecules³⁻⁵, and the nature of antigen recognition by these cells is unknown. To study antigen recognition by $\gamma\delta^+$ lymphocytes we raised a $\gamma\delta^+\alpha\beta^-$ -CD4⁻CD8⁻ line from an individual immune to PPD (purified protein derivative). This line showed a specific proliferative response to PPD and to a recombinant mycobacterial heat-shock protein (HSP) of relative molecular mass 65,000 (65K)⁶. The $\gamma\delta^+$ line was shown to exhibit a major response to HSP in the presence of autologous antigen-presenting cells (APCs). Minor responses occurred, however, with APCs matched for some HLA class I or II antigens, whereas no response occurred with HLA-mismatched APCs. These findings, therefore, document the requirement of HSP-reactive $\gamma\delta^+$ lymphocytes for histocompatible APCs.

To elucidate the participation of $\gamma\delta^+$ T cells in the immune response to antigen, we cultured peripheral-blood mononuclear cells (PBMCs) from a BCG-immune individual with PPD for six days. We analysed, by flow cytometry, the change in the percentage of TCR $\gamma\delta^+$ lymphocytes after staining with antibody directed at the δ -chain of the $\gamma\delta$ heterodimer. The results revealed a fourfold increase from the baseline of 4% $\gamma\delta^+$ of the CD3 $^+$ T cells in the unstimulated PBMCs to 16% after stimulation with PPD (data not shown). This expansion of CD3 $^+$ $\gamma\delta^+$ lymphocytes indicates either an antigen-specific stimulation of these lymphocytes, or a non-specific bystander and passive expansion of these cells in response to growth factors produced by the predominant $\alpha\beta^+$ cells. We therefore designed experiments to discover if an antigen-specific expansion of $\gamma\delta^+$ T cells occurs.

To isolate antigen-reactive $\gamma\delta^+$ T cells we raised a PPD-reactive line by stimulating PBMCs with PPD and restimulating with antigen and fresh feeders for an additional three-day period.

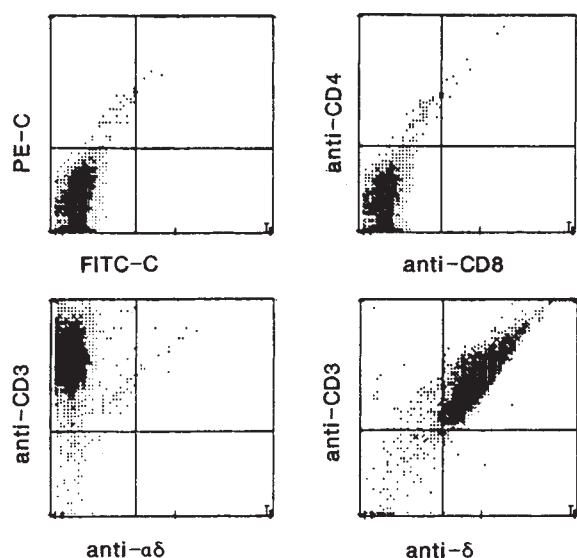


FIG. 1 Phenotypic analysis by double-colour fluorescence-activated cell sorting (FACS) of PPD-reactive CD4 $^+$, CD8 $^+$ and $\alpha\beta^+$ lymphocyte-depleted GD line. GD cells were double stained with anti-CD8-PE (phycoerythrin) antibody and anti-CD4-FITC (fluorescein), or with anti-CD3-PE and either anti- $\alpha\beta$ or anti- δ antibody with a second layer FITC-labelled anti-mouse antibody. Controls were labelled with isotype-matched antibodies.

METHODS. PBMCs from blood drawn from a healthy PPD-reactive individual were separated by sodium diatrizoate/Ficoll centrifugation and then cultured at $2 \times 10^6 \text{ ml}^{-1}$ in complete medium (RPMI 1640 plus 15% autologous serum plus $100 \mu\text{g ml}^{-1}$ streptomycin and 100 U ml^{-1} penicillin) together with $10 \mu\text{g ml}^{-1}$ PPD (Statens Seruminstitut, Copenhagen) in a 25-cm 2 flask (Costar) and incubated at 37 °C in a 5% CO $_2$ /95% air. On day 6, viable cells were separated by density-gradient centrifugation and recultured with fresh autologous irradiated (4,000 rad) PBMC feeders and PPD. On day 9, viable cells were recovered and CD4- and CD8-positive cells depleted. Briefly, the cells were incubated with supernatants from OKT4 and OKT8 hybridomas for 30 min at 4 °C and then washed and further incubated with goat-anti-mouse-antibody coated latex-polymer beads with a magnetic core (Advanced Magnetics) at 37 °C, on a gyratory shaker for 20 min. Subsequently, CD4- and CD8-positive cells bound to the beads were removed by a magnet (BioMag Separator, Advanced Magnetics). The remaining cells were washed and cultured with irradiated autologous feeders ($1 \times 10^6 \text{ ml}^{-1}$), PPD, and 10% T-cell growth-factor-containing media (Lymphocult, Biotest AG, Frankfurt). This cycle was repeated every 7 days and the expanded cells were depleted of CD4- and CD8-positive cells repeatedly, together with an anti- $\alpha\beta$ (BMA 031) 18 antibody for depletion of $\alpha\beta$ lymphocytes. Phenotypic marker analysis was determined by standard double-colour FACS. The monoclonal antibodies used were anti-Leu-4-PE (Becton) for the CD3 marker, anti-Leu-3a-PE for the CD4 marker, anti-Leu-2a for the CD8 marker, BMA 031 for $\alpha\beta$, and TCR $\delta 1$ (ref. 20) for $\gamma\delta$. For the unlabelled antibodies a second layer of labelled goat-anti-mouse-FITC (Becton) was applied before FACS analysis. Controls were run with isotype-matched antibodies.

Subsequently, we enriched for $\gamma\delta^+$ cells by negatively depleting CD4 $^+$ and CD8 $^+$ lymphocytes; we incubated the line with anti-CD4 and anti-CD8 monoclonal antibodies and removed the CD4 $^+$ and CD8 $^+$ cells with anti-mouse immunoglobulin-coated latex beads. Such CD4- and CD8-depleted cells were then expanded by incubating with fresh APCs, PPD and T-cell growth-factor-containing media. Further serial depletion of this line with anti-CD4 and anti-CD8 antibody followed by an anti- $\alpha\beta$ antibody resulted in a homogeneous and stable $\gamma\delta^+/\alpha\beta^-$ -CD3 $^+$ CD4 $^-$ CD8 $^-$ line (GD line) as revealed by double staining with anti-CD3 antibody and the appropriate anti-TCR antibodies, or double staining with anti-CD4 and anti-CD8 antibodies (Fig. 1). Furthermore, immunoprecipitation of [^{125}I]-labelled GD cells with anti-TCR $\delta 1$ and TCR C γ antibody revealed a 40K TCR δ -chain and a 55K TCR γ -chain, respectively (Fig. 2). The δ -subunit showed a marked shift in mobility under reducing and non-reducing conditions consistent with previous reports for $\gamma\delta^+$ T cells 2 . These cells were also shown, by flow cytometry, to express CD2 (sheep red blood cell receptor), UCHL-1, the activation antigens HLA-DR and the interleukin-2 (IL-2) receptor, but lacked CD45R and CD16 (Fc γ receptor and a natural killer-cell marker) antigens. The purity of the line in terms of function was confirmed by incubating the line with monoclonal antibodies directed against components of the TCR complex, specifically, CD3, the δ -chain and the $\alpha\beta$ complex, in the presence of plastic-adherent APCs.

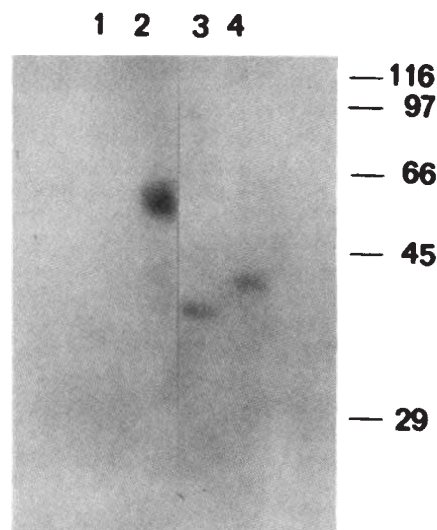


FIG. 2 Immunoprecipitation of γ and δ from the GD line. Immunoprecipitations from [^{125}I]-labelled GD cell lysates were resolved by SDS-PAGE (10% acrylamide) under reducing and non-reducing conditions as previously described 20 . Size markers, relative molecular mass in thousands. Lane 1, control lane with protein A-Sepharose and second anti-mouse-antibody (non-reducing); lane 2, γ (non-reducing) immunoprecipitated using an anti-C γ antibody; lane 3, δ (non-reducing); and lane 4, δ (reducing) immunoprecipitated using anti- $\delta 1$ antibody.

METHODS. GD cells were radio-iodinated by the lactoperoxidase technique and then lysed in 4 ml of lysing buffer (10 mM Tris pH 8.0, 140 mM NaCl, 0.3% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulphonate (CHAPS), 2 mM phenylmethylsulphonyl fluoride and 8 mM iodoacetamide). An aliquot of the lysate was boiled with 1% SDS for 3 min to dissociate the $\gamma\delta$ -CD3 complex. The lysate was diluted with Triton X100 to give a final concentration of 1% Triton and 0.1% SDS and then precleared with protein A-Sepharose CL-4B coated with anti-mouse antibody. Precleared lysates were immunoprecipitated using anti- $\delta 1$ or anti-C γ (both a gift of Dr M. B. Brenner) and the antigen-antibody complexes collected overnight with anti-mouse-antibody coated protein A-Sepharose. The immune complexes were washed in the lysing buffer containing 1% Triton and 0.1% SDS and then boiled in sample buffer containing 3% SDS, 20% glycerol and 0.125 M Tris (pH 6.8). For reducing samples 2 mM dithiothreitol was also included in the sample buffer. All the samples were incubated with 10 mM iodoacetamide for 10 min at 23 °C before analysis by SDS-PAGE.

The results (data not shown) showed the line to proliferate in response to either anti-TCR $\delta 1$ or anti-CD3 but not to anti- $\alpha\beta$ antibodies, providing further evidence that the GD line is composed only of $\gamma\delta^+$ cells.

The specific functional capability of the GD line was tested by a proliferative assay against PPD and an irrelevant antigen. For such studies, GD cells were cultured together with autologous APCs in the presence of either PPD, formalin-inactivated *Candida* antigen, or media alone. The results (Fig. 3a) showed a marked proliferative response to PPD but essentially no response to *Candida* antigen, reflecting the specificity of these $\gamma\delta^+$ lymphocytes to PPD. These findings, therefore, provide evidence that $\gamma\delta^+$ T cells do recognize antigen specifically, as do $\alpha\beta^+$ lymphocytes. Because PPD is a crude culture filtrate of *Mycobacterium bovis* strain BCG and contains several tuberculin proteins, we obtained a well-characterized and purified mycobacterial antigen better to assess antigen specificity. We therefore tested the response of the GD line to purified recombinant 65K HSP cloned from BCG⁶. The line demonstrated a vigorous response to this antigen (Fig. 3b) indicating the 65K mycobacterial HSP to be an important inducer of $\gamma\delta^+$ T cells.

The CD4 and CD8 cell surface markers, which are TCR-associated MHC (major histocompatibility complex) co-recognition molecules were not present on the GD line. We were interested, therefore, in finding out if $\gamma\delta^+$ -CD4⁻CD8⁻ lym-

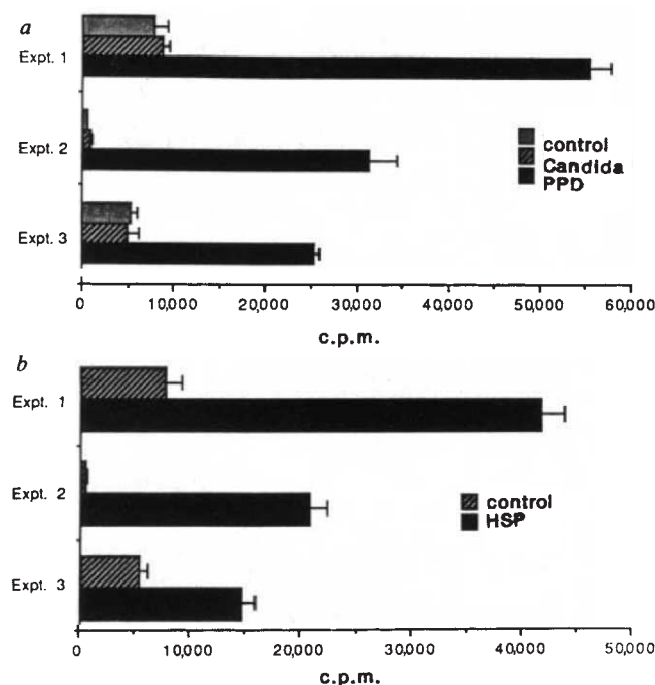


FIG. 3 Specificity of GD line to PPD (a) and response to 65K mycobacterial HSP (b). GD cells were cultured with plastic-adherent APCs with either PPD, HSP, *Candida* or with no antigen added in the controls. Proliferation was assessed by the incorporation of [³H]thymidine and the results expressed as mean counts per minute (c.p.m.) $\pm$ s.d. of triplicates.

METHODS. To test for antigen reactivity, GD cells that had retained the $\gamma\delta^+\alpha\beta^-$ /CD4⁻CD8⁻ phenotype for weeks and seven days after their last stimulation with antigen were incubated with IL-2-containing media feeders and 1×10^4 irradiated (4,000 rad) autologous plastic-adherent monocytes as APCs, which were distributed in each well of a 96-well flat-bottom microtitre plate (Falcon). PPD at 10 mg ml^{-1} , *Candida* at 1×10^6 formalin-treated yeast cells per well, HSP at $10 \mu\text{g ml}^{-1}$ were added to the wells in triplicates. Control cultures without antigen were included. The cells were incubated for 72–96 h and pulsed with $1.0 \mu\text{Ci}$ [³H]thymidine per well for the final 12 h before collection on glass-fibre filters using an automatic cell collector. [³H]TDR incorporation was assayed by liquid scintillation counting.

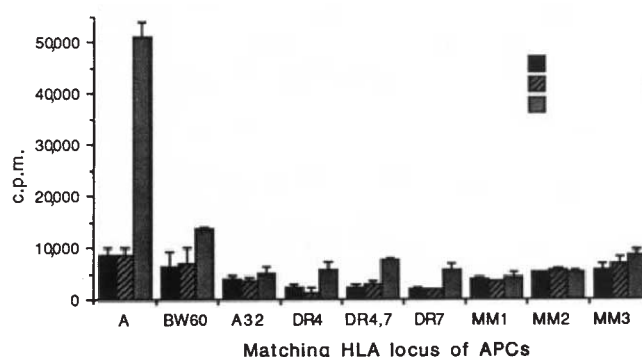


FIG. 4 HLA restriction pattern and specificity of the GD line to HSP. The results are expressed in mean c.p.m. per triplicate well of [³H]TDR incorporation of cultures containing HSP, *Candida* or no antigen in the presence of APCs from different sources. GD cells as described above were cultured in the presence of plastic-adherent APCs from the following HLA-typed sources: autologous (A32, Bw60, Bw63, DR4, DR7), HLA class I locus-matched (A2, A9, Bw60, Cw1, Cw3, DRw9, DQw3 and A2, A32, B35, Bw61, Cw4, DR5, DRw8, DQw1), HLA class II locus-matched (A2, A11, B7, Cw1, Cw3, DR4, DRw9, DQw3; A1, A24, B18, Bw44, DR4, DR7; A2, A26, B5, Bw4, Bw6, DR3, DR7), and HLA-mismatched (MM1–MM3) (A11, A24, B27, B35, Cw2, Cw4, DR2, DQw1; A1, A2, B8, Bw41, DR3; A1, A2, B8, B15, DR3, DR6).

phocytes also require MHC antigens to recognize antigen on accessory cells. To address this important question the GD line was cultured with APCs, from various individuals, that were matched with the line at some HLA class I or II loci, or were completely mismatched to HLA in the presence or absence of either HSP or *Candida* antigen. The line showed a vigorous proliferative response to HSP in the presence of autologous APCs, but a relatively minor response to HSP when the allogeneic APCs were matched at some HLA class I or DR loci (Fig. 4). No responses were found, however, when the APCs were mismatched to HLA. These findings therefore reveal these HSP-reactive $\gamma\delta^+$ T cells to be primarily self restricted. The minor responses to HSP in the presence of APCs with matched HLA loci may imply restriction in the HLA region. Because these responses were significantly lower than the response with autologous APCs, however, the data obtained suggest the presence of a molecule which is not detected by conventional HLA typing.

The role of $\gamma\delta^+$ T cells in the immune system has been a mystery since the discovery of these cells. It has been speculated that $\gamma\delta^+$ T cells are functionally similar to $\alpha\beta^+$ T cells, but are present only in smaller numbers. The data presented here provide evidence that $\gamma\delta^+$ T cells do indeed recognize antigens that are also recognized by $\alpha\beta^+$ lymphocytes, and also suggest the possibility of the participation of these cells in the immune response to infectious agents. The response of our line to the 65K mycobacterial HSP, which has been shown to be a major immunogen for both CD4⁺ (refs 8–11) and CD8⁺ (ref. 12) T cells as well as B cells¹³, provides further proof for a possible distinct role played by $\gamma\delta^+$ T cells in immunity. HSPs are highly conserved proteins that are widely distributed in nature¹⁴ and are synthesized in response to sudden increases⁴ in temperature or other environmental stresses¹⁵. It has recently also been suggested that murine $\gamma\delta^+$ dendritic epidermal cells might recognize mammalian HSPs stimulated by cell damage and therefore such cells may be involved in the elimination of damaged and stressed cells¹⁶. Because eukaryotic and prokaryotic HSPs share antigenic properties, it is possible to speculate that the same immune cell that has evolved to eliminate damaged cells in homeostasis, may have taken the additional role of host protection by also recognizing similar antigens from a microbial invader. The demonstration, by limiting-dilution analysis, of UCHL1⁺/CD45R⁻ cells as recall antigen-responsive T cells¹⁷, suggests however, that these 65K HSP-reactive UCHL1⁺/CD45R⁻/ $\gamma\delta^+$

cells may be involved in memory rather than serving as a natural and unprimed component of the immune system responding to a 'superantigen'¹⁸, as has recently been suggested for some powerful microbial T-cell stimulants. Further studies are necessary to evaluate the role of these cells in immunity and pathogenesis. □

Note added in proof. After submission of this paper, Holoshitz *et al.*²¹ reported an HSP-responsive $\gamma\delta$ T-cell clone, isolated from a patient with rheumatoid arthritis, and Modlin *et al.*²² isolated $\gamma\delta$ T cells from patients with leprosy.

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- Weiss, A. *et al.* *A. Rev. Immun.* **4**, 593-619 (1986).
- Brenner, M. B., Stromminger, J. L. & Krangel, M. S. *Adv. Immun.* **43**, 133 (1988).
- Brenner, M. B. *et al.* *Nature* **322**, 145-149 (1986).
- Bank, I. *et al.* *Nature* **322**, 179-181 (1987).
- Borst, J. *et al.* *Nature* **325**, 683-688 (1987).
- Thole, J. E. R. *et al.* *Infect. Immunity* **50**, 800-806 (1985).

- Swain, S. I. *Immun. Rev.* **74**, 129-143 (1983).
- Emmrich, F., Thole, J. E. R., van Embden, J. & Kaufman, S. H. E. *J. exp. Med.* **163**, 1024-1029 (1986).
- Kaufman, S. H. E., Vath, U., Thole, J. E. R., Van Embden, J. D. A. & Emmrich, F. *Eur. J. Immun.* **17**, 351-357 (1987).
- Ottenhoff, T. H. M., Kale, A. B., van Embden, J. D. A., Thole, J. E. R. & Kiessling, R. *J. exp. Med.* **168**, 1947-1952 (1988).
- Oftung, F. *et al.* *J. Immun.* **141**, 2749-2754 (1988).
- Rees, A., Scoging, A., Mehlert, A., Young, D. B. & Ivanyi, J. *Eur. J. Immun.* **18**, 1881-1887 (1988).
- Britton, W. J., Hellquist, L., Basten, A. & Inglis, A. S. *J. exp. Med.* **164**, 695-708 (1986).
- Schlessinger, M. J., Ashburner, M. & Thessiers, A. (eds). *Heat Shock from Bacteria to Man* (Cold Spring Harbor Laboratory, New York, 1982).
- Linquist, S. A. *Rev. Biochem.* **55**, 1151-1191 (1986).
- Asarnow, D. M. *et al.* *Cell* **55**, 837-847 (1988).
- Merkenschlager, L. T., Edwards, R. & Beverly, C. L. *Eur. J. Immun.* **18**, 1653-1661 (1988).
- White, J. *et al.* *Cell* **56**, 27-35 (1989).
- Lanier, L. L., Ruitenberg, J. J., Allison, J. P. & Weiss, A. in *Leukocyte Typing III* (eds McMichael, A. J. *et al.*) 175-178 (Oxford University Press, 1987).
- Brenner, M. B. *et al.* *Nature* **325**, 689-694 (1987).
- Holoshitz, J., Koning, F., Coligan, J. E., De Bruyn, J. & Strober, S. *Nature* **339**, 226-229 (1989).
- Modlin, R. L. *et al.* *Nature* **339**, 544-548 (1989).

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Preferential germline mutation of the paternal allele in retinoblastoma

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THE event triggering malignant proliferation in 70% of retinoblastoma tumours is loss of heterozygosity for chromosome 13q14, whereby the normal retinoblastoma gene (RB1) allele is lost and an already mutated RB1 allele remains in the tumour¹⁻³. The first allele suffers a mutational event—deletion, duplication or point mutation (manuscript in preparation)—either in the germ line (all bilateral patients) or in a somatic retinal cell (most unilateral patients). Most bilateral patients have no family history of retinoblastoma and are presumed to have new germline mutations which arose in the egg, sperm or early embryo. We have determined the parental origin of the retained allele in nine retinoblastoma tumours from eight unrelated non-familial cases by using RB1-linked genetic markers. Six tumours retained the paternal allele and three retained the maternal allele. Of the three unilateral tumours, only one retained the paternal RB1 allele. Thus, there is no evidence that the paternal RB1 allele is preferentially retained in retinoblastoma, as has been suggested to be the case in osteosarcoma^{4,5}. By contrast, tumours from four of the five bilateral patients retained the paternal RB1 allele. This suggests either that new germline RB1 mutations arise more frequently during spermatogenesis than during oogenesis, or that imprinting in the early embryo affects chromosomal susceptibility to mutation.

In two types of cancer a preference has been documented for retention of the paternal chromosome during the process of loss of heterozygosity (LOH). Osteosarcoma, the most common second malignancy in patients with germline RB1 mutations⁶⁻⁸, shows frequent LOH for chromosome 13 and a preference for retention of the paternal chromosome^{4,5,9}. In Wilms tumours the paternal chromosome 11p is preferentially retained during LOH¹⁰⁻¹³.

These investigators interpreted their results in terms of genomic imprinting in the tumour, whereby retention of the paternal chromosome may be required for survival of an osteosarcoma cell. In contrast to RB tumours with near-diploid karyotypes¹⁴, osteosarcoma tumours frequently are aneuploid^{5,9}, complicating the interpretation of LOH. Because 7 of the 13 osteosarcomas arising by somatic mutations reported by Toguchida *et al.*⁴ had deletions within RB1 alleles, only 6

tumours could be rigorously evaluated for significance of the apparent LOH. One retained the maternal allele and 5 retained the paternal allele. This difference is not statistically significant.

To determine whether there is a preferential retention of either allele in RB, we studied RB tumours for LOH. Twenty-seven informative tumours were found by screening 36 RB tumours from 16 bilateral and 17 unilateral patients. Of these, sixteen tumours from 7 bilateral and 8 unilateral patients showed LOH (59%), which is consistent with previous studies^{2,15,16}.

The parental origin of the chromosome retained in the tumours could be determined for 12 of the 15 patients showing LOH. Four cases were excluded: two had inherited a mutant RB1 allele from their affected fathers, and two tumours showed LOH for only one restriction fragment length polymorphism, which may indicate only a small deletion in this region. Therefore, the data for 9 RB tumours from 8 non-familial patients could be included in the analysis for the parental origin of the alleles retained (Fig. 1). These tumours had the near-diploid karyotypes characteristic of RB¹⁴.

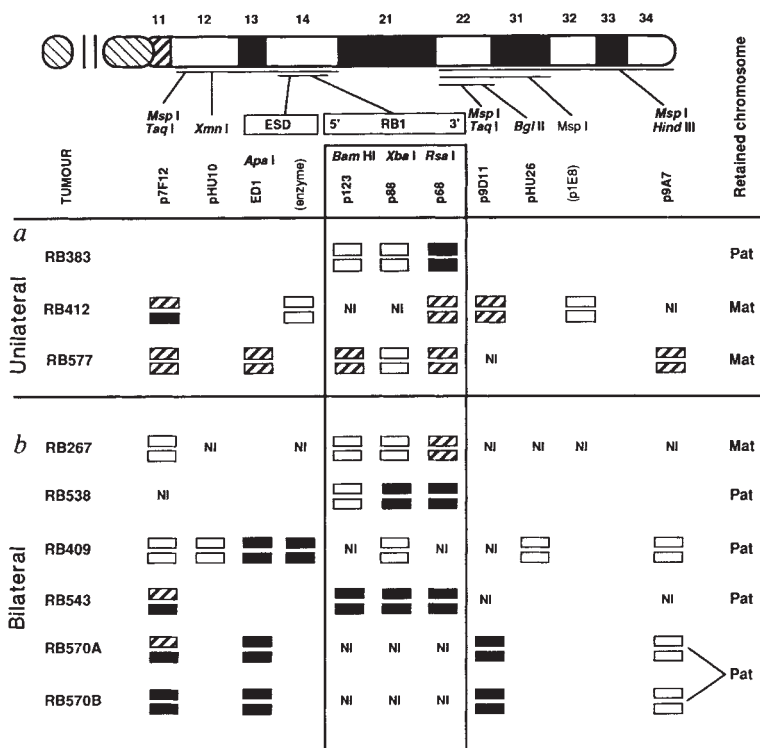
Three tumours retained the maternal allele, whereas six tumours from five patients retained the paternal allele. Two of the three unilateral tumours (Fig. 1a) retained the maternal allele and one the paternal allele, showing that either allele can be retained in RB tumours. Our data clearly indicate that imprinting within or near RB1 plays no role in RB tumours.

The five bilateral patients had new germline mutations, three of which were confirmed by identification of the germline mutation (manuscript in preparation). The tumours of four of these patients retained the paternal allele (Fig. 1b). However, one tumour (RB267) retained the maternal allele, indicating that new germline mutations can occur on both maternal and paternal chromosomes.

Combining our data with similar data reported by Dryja *et al.*¹⁷, shows a significant difference in the parental origin of the chromosome retained in bilateral RB tumours, but not in unilateral ones. Thirteen out of 14 bilateral tumours retained the paternal chromosome ($0.001 < P < 0.01$). In contrast, four of 10 unilateral tumours retained the paternal chromosome, not a significant difference ($0.5 < P < 0.7$). Two important conclusions are evident. First, there is no selection in the somatic cells for retention of the maternal or paternal alleles, consistent with other data on RB. Both alleles make transcripts in RB tumours¹⁸ and family studies show equal penetrance by RB1 mutations inherited from the mother or the father¹⁹. Second, germline RB1 mutations seem to arise more frequently on the paternal allele than on the maternal allele. This may indicate either that mutation of RB1 is more common during spermatogenesis than oogenesis as a result of differences between male and female meiosis, DNA methylation, or environmental exposure; or that the paternal chromosome in the early embryo is more at risk for mutation, or deficient in DNA repair. □

FIG. 1 Parental origin of the retained chromosome 13 in RB. The approximate loci of the RFLP markers on chromosome 13 is indicated at the top. a, Unilateral; b, bilateral. The two markers for which only previously published data are included^{2,3}, are bracketed. NI, non-informative for LOH. Boxes: diagonal stripes, maternal allele; black, paternal allele; white, LOH but NI for parental origin. Identified RB1 mutations are as follows: RB543 germline, 10-bp deletion exon 18; RB570 germline, 9-bp deletion exon 19; RB538 germline, 55-bp duplication exon 10 (J.M.D. *et al.*, in preparation).

METHODS. RB tumour DNA was obtained from surgical specimens, supplemented by xenografts in immune-deficient mice²⁰ and/or in tissue culture²¹. Constitutional DNA of the patients and parents was obtained from lymphoblasts of lymphoblastoid cell lines. DNA was prepared by a standard method²². The probes used in this study were generously provided by T. Dryja²³, W. K. Cavenee²⁴ and J. Squire²⁵. All probes were labelled with [α -³²P] dCTP by random primer labelling²⁶ to an activity of $4\text{--}6 \times 10^5$ c.p.m. μg^{-1} DNA. DNA samples were digested with appropriate restriction enzymes, electrophoresed in 0.7–1.0% agarose gel and transferred to Zeta-probe or Hybond membranes²⁷. Hybridization was performed as described previously²⁸. After hybridization, filters were routinely washed twice at room temperature in $2 \times \text{SSC}$, 0.1% SDS and once under high stringency conditions in $0.1 \times \text{SSC}$, 0.1% SDS at 65 °C. Autoradiographs were developed after 24–48 h at –70 °C.



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- Knudson, A. G. *Proc. natn. Acad. Sci. U.S.A.* **68**, 820–823 (1971).
- Cavenee, W. K. *et al. Nature* **305**, 779–784 (1983).
- Godbout, R., Dryja, T. P., Squire, J., Gallie, B. L. & Phillips, R. A. *Nature* **304**, 451–453 (1983).
- Toguchida, J. *et al. Nature* **338**, 156–158 (1989).
- Toguchida, J. *et al. Cancer Res.* **48**, 3939–3943 (1988).
- Abramson, D. H., Ellsworth, R. M., Kitchin, F. D. & Tung, G. *Ophthalmology* **91**, 1351–1355 (1984).
- Draper, G. J., Sanders, B. M. & Kingston, J. E. *Br. J. Cancer* **53**, 661–671 (1986).
- Roarty, J. D., McLean, I. W. & Zimmerman, L. E. *Ophthalmology* **95**, 1583–1587 (1988).
- Hansen, M. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 6216–6220 (1985).
- Orkin, S. H., Goldman, D. & Sallan, S. E. *Nature* **309**, 172–174 (1984).
- Reeve, A. E. *et al. Nature* **309**, 174–176 (1984).
- Schroeder, W. T. *et al. Am. J. hum. Genet.* **40**, 413–420 (1987).
- Wilkins, R. J. *Lancet* **i**, 329–331 (1988).
- Squire, J., Gallie, B. L. & Phillips, R. A. *Hum. Genet.* **70**, 291–301 (1985).
- Benedict, W. F. *et al. Cancer Res.* **47**, 4189–4191 (1987).
- Dryja, T. P. *et al. N. Engl. J. med.* **310**, 550–553 (1984).

- Dryja, T. P. *et al. Nature* **339**, 556–558 (1989).
- Dunn, J. M., Phillips, R. A., Becker, A. J. & Gallie, B. L. *Science* **241**, 1797–1800 (1988).
- Matsunaga, E. *Hum. Genet.* **62**, 124–128 (1982).
- Gallie, B. L., Albert, D. M., Wong, J. J. Y., Buyukmickci, N. & Puliafito, C. A. *Invest. Ophthalmol.* **16**, 256–259 (1977).
- Gallie, B. L., Holmes, W. & Phillips, R. A. *Cancer Res.* **42**, 301–305 (1982).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Wiggs, J. *et al. N. Engl. J. Med.* **318**, 151–157 (1988).
- Cavenee, W., Leach, R., Mohandas, T., Pearson, P. & White, R. *Am. J. hum. Genet.* **36**, 10–24 (1984).
- Squire, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 6573–6577 (1986).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **132**, 6–13 (1983).
- Reed, K. C. & Mann, D. A. *Cancer Res.* **37**, 1003–1010 (1985).
- Goddard, A. D. *et al. Molec. cell. Biol.* **8**, 2082–2088 (1988).

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A role for calpactin in calcium-dependent exocytosis in adrenal chromaffin cells

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STIMULATION of bovine adrenal chromaffin cells results in a rise in the concentration of cytosolic calcium^{1–3} which triggers the release of catecholamines by exocytosis. Several cytosolic proteins that bind to secretory granule membranes in a calcium-dependent manner have been implicated in exocytosis^{4–12} and some belong to a family of calcium-binding proteins, the annexins⁶. One of these, calpactin, is a tetramer consisting of two heavy and two light chains (relative molecular masses 36,000 and 10,000 respectively)^{7,8} and can aggregate^{10,11} and fuse membranes *in vitro* in

the presence of arachidonic acid¹⁰. Calpactin is found at the cell periphery^{13,14} and is phosphorylated when chromaffin cells are stimulated¹⁵. We show here that both calpactin and calpactin heavy chain (p36) reconstitute secretion in permeabilized chromaffin cells in which secretion has been reduced as a result of leakage of cellular components. This effect is inhibited by an affinity-purified antibody against p36. Secretion from permeabilized cells is inhibited by a synthetic annexin-consensus peptide, but not by a nonspecific hydrophobic peptide; this inhibition is reversed by p36. Our results indicate that either calpactin or p36 is essential for exocytosis.

Chromaffin cells permeabilized with digitonin are stimulated to secrete by micromolar concentrations of calcium¹⁶. If, after permeabilization, cells are maintained at low calcium concentrations, they leak proteins that are essential for exocytosis¹⁷. Here we permeabilized chromaffin cells for 25 min before stimulation with calcium, when secretion is reduced to 30% of that by cells that have not been pre-permeabilized; the effects of exogenous factors which might reconstitute secretion or inhibit residual secretion could then be tested. In these conditions, calpactin leaks out of the cells (data not shown) so we investigated the effects of incubating calpactin and p36 purified from bovine lung¹⁸ with permeabilized cells (Fig. 1a): we found a 2–3-fold increase in secretion when the cells were subsequently stimulated

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by 10 μ M calcium after permeabilization for 25 min. This stimulation was detectable with as little as 0.5 μ g per well of added protein. The extent of secretion ($\sim 30\%$ of total catecholamine) was essentially the same as that from cells that had not been pre-permeabilized. Therefore, calpactin and p36 could completely reconstitute secretion in permeabilized cells following leakage of cellular components. Moreover, the calpactin heterotetramer is not necessary for reconstitution as p36 is sufficient on its own. Catecholamine release stimulated by p36 is due to exocytosis, rather than to release following intracellular granule-granule fusion. The granule protein dopamine β -hydroxylase, which is not released after granule-granule

fusion¹⁹, is co-released with catecholamine in response to p36 at 10 μ M calcium ($210 \pm 20\%$ and $185 \pm 33\%$ increase in catecholamine and dopamine β -hydroxylase release respectively, in the presence of p36).

In contrast to the reconstitution of secretion by calpactin and p36, the crude calcium-dependent membrane-binding fraction from adrenal medulla^{5,12} has no effect on secretion (at ≤ 50 μ g protein per well), neither does a 100,000g supernatant of whole adrenal medulla (≤ 600 μ g protein per well). It is possible that these crude mixtures could contain inhibitors of p36 activity. Figure 1b shows that p36 does not affect the calcium dependence of exocytosis but increases the amount of secretion at higher calcium concentrations. We tested the specificity of the reconstitution by p36 by incubating p36 with affinity-purified antibody against p36^{14,20} before adding it to cells. The antibody has no effect on secretion, but it completely inhibits the reconstitution of secretion by p36, showing that the effect is specific to p36 and is not attributable to a minor contaminant in the p36 fraction (Fig. 2).

The residual secretion present before re-addition of calpactin or p36 could be due to p36 still present in cells after permeabilization for 25 min (data not shown). In addition, p36 does not stimulate secretion at 0.3 μ M or 1 μ M calcium, which could

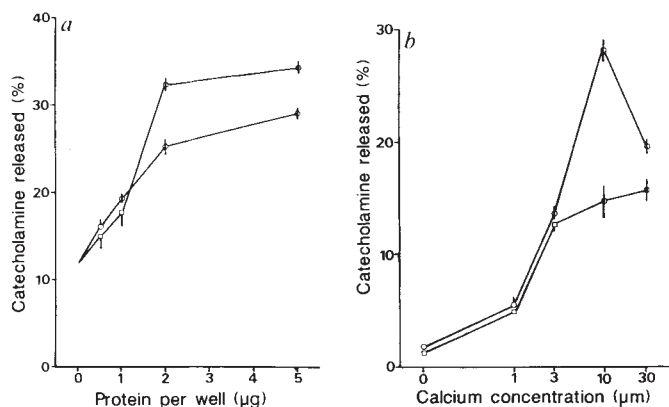


FIG. 1 Reconstitution of calcium-dependent exocytosis by purified calpactin and p36 in permeabilized adrenal chromaffin cells. *a*, Concentration-dependence of the increase in secretion in pre-permeabilized cells due to calpactin (○) or p36 (□) in response to 10 μ M calcium. *b*, Calcium-dependence of the secretion in control cells (□) and cells incubated with 4 μ g per well of p36 (○). Data are shown as mean $\pm$ s.e.m. ($n=4$).

METHODS. Bovine adrenal chromaffin cells were isolated, plated at 800,000 cells per well in 24-well trays, and cultured for 3–7 days²². Cells were permeabilized by 20 μ M digitonin in 139 mM potassium glutamate, 2 mM ATP, 2 mM magnesium chloride, 5 mM EGTA and 20 mM HEPES, pH 6.5. After an initial 10 min permeabilization phase, the cells were incubated at 300 μ l per well in permeabilization buffer containing appropriate concentrations of calpactin or p36 (purified as described in ref. 18) for a further 15 min. Cells were stimulated to secrete in fresh permeabilization buffer (300 μ l per well) containing CaCl_2 . Aliquots of the medium were taken for catecholamine assay after 15 min. Released catecholamine was expressed as a percentage of total catecholamine content²³. All incubations were at 20 $^\circ\text{C}$.

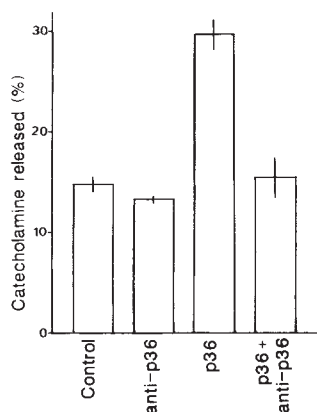


FIG. 2 Reconstitution of secretion by p36 neutralized by affinity-purified antibody against p36. Data are shown as mean $\pm$ s.e.m. ($n=4$).

METHODS. Chromaffin cells were prepared, permeabilized and treated as described in the legend to Fig. 1. Affinity-purified p36 antibody was prepared, affinity-purified by chromatography on p36-Sepharose and characterized as previously described²⁰. Permeabilized cells were incubated with 4 μ g per well of p36, or with 4 μ g per well of p36 pre-incubated with anti-p36 (1:50) at 20 $^\circ\text{C}$ for 1 h.

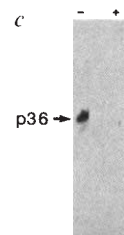
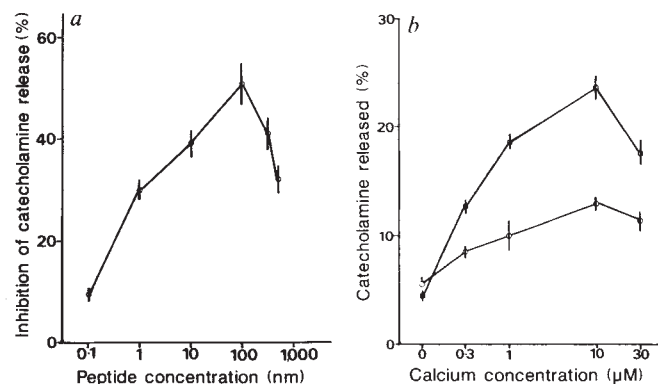


FIG. 3 Inhibition of exocytosis in permeabilized adrenal chromaffin cells and p36 binding to membranes by annexin-consensus peptide. *a*, Dose-dependent inhibition of secretion by annexin-consensus peptide. *b*, Calcium-dependence of secretion from control cells (□) and cells incubated with the annexin-consensus peptide (○). Data are shown as mean $\pm$ s.e.m. ($n=4$). *c*, Binding of p36 to chromaffin granule membranes in the absence (–) or presence (+) of 100 nM peptide.

METHODS. Chromaffin cells were prepared and permeabilized as described in the legend to Fig. 1. After the initial 10 min permeabilization, cells were incubated with peptide (100 nM in *b*) for 15 min before stimulation by calcium (10 μ M in *a*, or as indicated in *b*). The level of catecholamine released after permeabilization varied with cell batch, but was particularly high here. The extent of binding of p36 to chromaffin granule membranes at 10 μ M calcium⁵ following a 30 min incubation with or without peptide was assessed by polyacrylamide gel electrophoresis of washed membranes and immunoblotting²⁰ with anti-p36. The annexin-consensus peptide (CAMKGAGTDEGALIEILASR; one-letter code) was synthesized on an Applied Biosystems peptide synthesizer and purified by HPLC.

result from activation of a separate pathway. To determine whether endogenous p36 is involved in the residual calcium-dependent exocytosis in permeabilized chromaffin cells, we used an annexin-consensus peptide, the sequence of which (CAMKGAGTDEGALIEILASR; one-letter code) is based on the conserved region of the annexin family of proteins^{6,21}, and which may act as a competitive antagonist against p36. Pre-incubation of permeabilized chromaffin cells with this peptide inhibits secretion when cells are subsequently stimulated with 10 μ M calcium (Fig. 3a), maximal inhibition being at a peptide concentration of 100 nM. The peptide only partially inhibits secretion, and the effect is reduced at higher concentrations of peptide, when it triggers calcium-independent catecholamine release. The sequence of the annexin-consensus peptide is different from synthetic peptides that, like the annexins, can inhibit phospholipase A₂ (ref. 22), indicating that the annexin-consensus-peptide is probably not acting by inhibition of phospholipase A₂. The inhibitory effect of the annexin-consensus-peptide is specific and is not a result of a shift in calcium requirement, as it is evident at all calcium concentrations (Fig. 3b). This implicates endogenous p36 in exocytosis triggered even at low calcium levels. Moreover, the inhibition is not due to a non-specific hydrophobic effect because another hydrophobic peptide (from the C terminus of human serum albumin, sequence LVAASQAALGL) gave no inhibition at comparable concentrations.

Inhibition of secretion by the annexin-consensus peptide was abolished by low concentrations (1 μ g protein) of p36. This suggests to us that the peptide may exert its effect by interfering with endogenous calpactin, perhaps by competing for calpactin or for p36-binding sites on the plasma or granule membrane. This possibility is supported by the prevention by the peptide (at 100 nM) of secretion reconstitution by increased levels of calpactin (at 4 μ g per well), which demonstrates that p36 and the peptide are in competition. The annexin-consensus peptide also inhibits binding of calpactin to purified chromaffin granule membranes in an *in vitro* assay (Fig. 3c).

Our results show that calpactin, or p36, is sufficient to reconstitute exocytosis in permeabilized chromaffin cells after a reduction in their secretory potential resulting from loss of cytosolic components. In addition, the inhibitory effect of the annexin-consensus peptide shows that endogenous calpactin, or a closely related protein, is essential for exocytosis. Calpactin (or p36) could therefore be the calcium-receptor protein involved in triggering calcium-dependent exocytosis in the adrenal chromaffin cell. □

Forward transport of glycoproteins on leading lamellipodia in locomoting cells

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IN several types of locomoting cells, active rearward transport of particles on the cell surface has been observed and correlated with motility¹⁻⁴. No forward transport of particles has previously been reported, however. Here we report rapid forward transport of concanavalin A-coated gold particles on the dorsal surfaces of lamellipodia of fish epidermal keratocytes. These movements are active, not diffusive, and more rapid than either rearward particle transport or the rate of cell locomotion. We observed forward transport in migrating, but not in stationary cells, and could block the movement by treatment with cytochalasin D. These studies demonstrate for the first time that small numbers of glycoproteins can be actively transported on the surface of the cell to the front of the lamellipodium. We suggest that this mechanism transports proteins involved in cell locomotion, such as proteins necessary for adhesion, and could also produce an extensile force.

Rearward transport of particles on the dorsal surface of locomoting cells may be related to various cytoskeletal phenomena, such as the concerted retrograde motion of actin arcs and surface glycoproteins in locomoting chick embryo fibroblasts⁵. Rearward motion of actin filaments has also been observed by fluorescence photobleaching⁶ and by video-enhanced DIC microscopy^{7,8}. Forward transport of material could also be necessary for locomotion. As the cell extends its leading edge, various specific proteins would be needed, both for construction of the lamellipodium and for functions such as adhesion to the substrate.

We coated small (40 nm in diameter) gold particles with the lectin concanavalin A (con A), which binds to various glycoproteins. Fish keratocytes displayed normal rearward transport of particles when large aggregates of con A-coated gold particles became bound to the lamellipodium. The aggregates moved steadily backwards, as previously observed in other cell types. By contrast, single 40-nm-diameter con A-coated gold particles primarily displayed two types of motion: random diffusion or rapid, directed movement towards the front of the cell. Using the nanometre-level motion-analysis technique of Gelles *et al.*⁹, we determined the positions of particles undergoing the two types of motion for each video frame (Fig. 1). Particles would sometimes alternate between random and forward motion, that is, randomly moving particles would suddenly make rapid excursions towards the front of the cell and would then return to random motion. In many cases, particles beginning forward movement would continue until they reached the leading edge (Fig. 3).

We observed at least 20 rapid forward movements of more than 2 μ m in 5 h of viewing lamellipodia with 2-3 particles on average in the region 2-5 μ m from the leading edge. Many more particles made shorter forward excursions. Forward transport of particles occurred with a mean velocity of 60 μ m min⁻¹; diffusing particles had diffusion coefficients in the range of 10⁻¹⁰ to 10⁻⁹ cm² s⁻¹. We saw no rapid movements towards the rear of the cell.

In a population of particles in random motion, there is a probability that a few particles will by chance seem to undergo

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1. Knight, D. E., Kesteven, N. T. *Proc. R. Soc.* **B218**, 177-199 (1983).
2. Burgoyne, R. D. *Biosci. Rep.* **4**, 605-611 (1984).
3. Burgoyne, R. D. & Cheek, T. R. *FEBS Lett.* **182**, 115-118 (1985).
4. Creutz, C. E. *et al. J. biol. Chem.* **258**, 14664-14674 (1983).
5. Geisow, M. J. & Burgoyne, R. D. *J. Neurochem.* **38**, 1735-1741 (1982).
6. Burgoyne, R. D. & Geisow, M. J. *Cell Calcium* **10**, 1-10 (1989).
7. Gerke, V. & Weber, K. *EMBO J.* **3**, 227-233 (1984).
8. Glenney, J. J. *biol. Chem.* **261**, 7247-7252 (1986).
9. Sudhof, T. C. *et al. Biochemistry* **23**, 1103-1109 (1984).
10. Drust, D. S. & Creutz, C. E. *Nature* **331**, 88-91 (1988).
11. Powell, M. A. & Glenney, J. R. *Biochem. J.* **247**, 321-328 (1987).
12. Burgoyne, R. D. *Nature* **331**, 20 (1988).
13. Greenberg, M. E. & Edelman, G. M. *Cell* **33**, 767-779 (1983).
14. Burgoyne, R. D. & Cheek, T. R. *Biosci. Rep.* **7**, 281-288 (1987).
15. Creutz, C. E. *et al. J. biol. Chem.* **262**, 18660-18668 (1987).
16. Dunn, L. A. & Holz, R. W. *J. biol. Chem.* **258**, 4989-4993 (1983).
17. Sarafian, T., Aunis, D. & Bader, M. F. *J. biol. Chem.* **262**, 16671-16676 (1987).
18. Glenney, J. R., Tack, B. & Powell, M. A. *J. Cell Biol.* **104**, 503-511 (1987).
19. Creutz, C. E. *J. Cell Biol.* **91**, 247-256 (1981).
20. Burgoyne, R. D., Cambray-Deakin, M. & Norman, K. M. *J. molec. Neurosci.* **1**, 47-54 (1989).
21. Geisow, M. J., Fritzsche, U., Hexham, J. M., Dash, B. & Johnson, T. *Nature* **320**, 636-638 (1986).
22. Miele, L. *et al. Nature* **335**, 726-729 (1988).
23. Burgoyne, R. D., Morgan, A. & O'Sullivan, A. J. *FEBS Lett.* **238**, 151-155 (1988).

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directed motion for a short time. We calculated the probability that the forward motions we observed were outliers in a population of randomly diffusing particles, and determined that the forward movements of particles could not be accounted for by simple diffusion ($P = 10^{-48}$; Fig. 1). Furthermore, quantitative analysis of the mean-square displacement as a function of time according to the method of H. Qian *et al.* (manuscript in preparation) showed that there was a non-random, directed component of the motion of the particles (Fig. 2). These results indicate that there is an active mechanism for forward transport which is coupled intermittently to membrane glycoproteins.

The forward transport of particles is different from rearward transport in several respects. The rapid forward movements are intermittent—they seldom last longer than 1 s and are often interrupted by periods of random movement. Rearward transport is steady and prolonged. Forward transport is typical of single particles and small aggregates, whereas larger aggregates tend to undergo rearward transport. Forward transport is much faster than rearward transport. The average velocity towards the forward edge for the particles we observed was $60 \mu\text{m min}^{-1}$ (Fig. 2); instantaneous velocities were often several times faster. This velocity is significantly greater than the velocity of cell movement ($\sim 30 \mu\text{m min}^{-1}$), allowing particles to catch up to

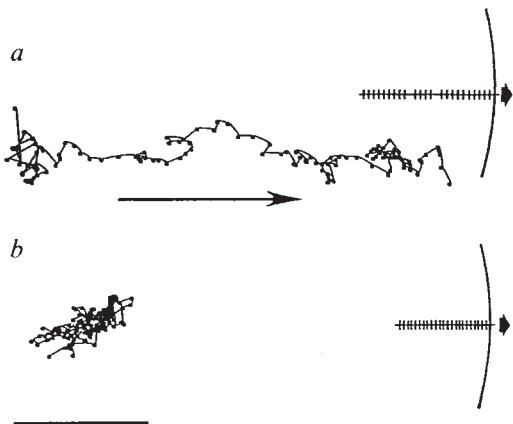


FIG. 1 Comparison of particle tracks for a transported particle versus a particle in random brownian motion. The positions of con A-coated gold particles are plotted every 1/30 s for a particle moving towards the leading edge (a) and for one undergoing random diffusion in a similar region of the cell (b). Long arrow, general direction of motion of the transported particle; short, thick arrows, direction of motion of the leading edge of the cell in each case; short vertical lines behind each arrowhead, successive positions of the leading edge over this time period, with the final position shown schematically by a long, curved line. Scale bar, $1 \mu\text{m}$.

We calculated the probability that a particle diffusing with diffusion coefficient D will traverse at least a certain distance in a given direction X simply by executing a random walk¹⁸. The equation $P(x) dx = (1/4\pi Dt) \exp(-x^2/4Dt) dx$ gives the probability that a particle initially at $x=0$ will diffuse to a location between x and $x+dx$ in time t . The probability that $x \geq x_0$ is $\int_{x_0}^{\infty} P(x) dx$, the error function $\text{erf}(x)$. Applying this analysis to the two particle trajectories in this figure (using a diffusion constant of $10^{-9} \text{ cm}^2 \text{ s}^{-1}$) shows that although trajectory *b* is consistent with purely random motion ($P > 0.5$), the probability that trajectory *a* resulted from simple diffusion is very small ($P < 10^{-9}$). Even an event with a very small probability will be expected to occur purely by chance if the period of observation is long enough. If P is the probability of the event occurring in one trial (as shown above), then the probability of this event occurring at least once in N trials is $1 - (1 - P)^N$. If the quantity 'particle-second' is defined as one particle observed for one second, then the total number of trials for each transport event is: (total period of observation of particles) (average number of particles in a video frame)/(the duration of the event) (one particle). Once the probability of observing each event is determined, the probability that all the events observed would occur in the same 5-h period purely by chance is the product of the individual probabilities. Applying this calculation to 15 events (in which the image quality and stability were sufficient for accurate position analysis), we obtained a probability of 10^{-48} that these events result from random motion.

METHODS. Fish epidermal keratocytes from goldfish (*Carassius auratus*) were cultured from whole-scale explants¹⁹ and grown in Ringer solution supplemented with Gibco amphibian culture medium²⁰. The cells were observed by video-enhanced DIC microscopy on a Zeiss IM-35 inverted microscope in Ringer's solution to which 40-nm-diameter gold particles coated with con A had been added. As the particles settled onto the dorsal surfaces of the cells, their motion was observed and recorded on videotape. Selected sequences were then copied onto video disk for computer analysis. Particle positions for each frame were determined using the method of Gelles *et al.*⁹.

an extending edge. In contrast, gold-particle aggregates and large plastic spheres ($0.5 \mu\text{m}$ diameter) were transported rearward at a steady rate of $15\text{--}30 \mu\text{m min}^{-1}$ relative to the substratum (data not shown).

Treatment with $2 \mu\text{g ml}^{-1}$ cytochalasin D inhibited motility within 2 min and stopped all transport of particles on the cell surface within 5 min; cytoplasmic organelle movements, presumably microtubule-based, persisted for more than 50 min. (Cytochalasin D disrupts actin filaments.) Note, however, that this result does not exclude a non-cytoskeletal mechanism, as there are potentially indirect effects of cytoskeleton disruption.

There are key differences between the approach used in our study and that of earlier studies which have made it possible to detect forward transport. Previous workers used large particles, typically $>0.2 \mu\text{m}$ in diameter, which would be expected to interact with and crosslink many membrane glycoproteins. Extensive crosslinking might initiate rearward transport, as happens, for example, in the capping of membrane proteins¹⁰. The individual 40-nm diameter particles used in this study do not activate rearward transport, perhaps because they crosslink only relatively few membrane proteins. The observation that larger aggregates of the same con A-coated gold particles were uniformly transported rearward supports this suggestion. A second difference is the choice of cell type. Fish epidermal keratocytes have large, featureless lamellipodia ($20\text{--}50 \mu\text{m}$ across) which provide a large surface area on which to observe particle motion.

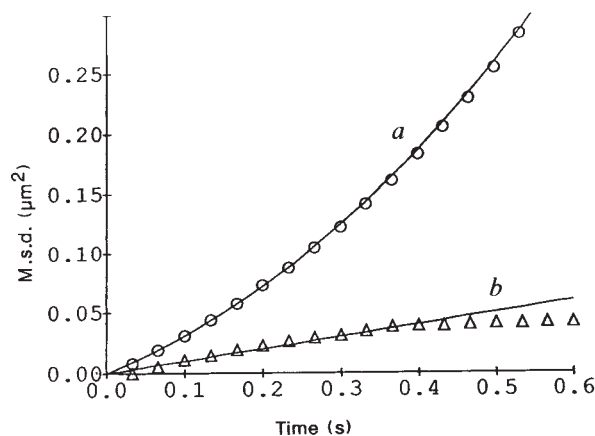


FIG. 2 Mean-square displacement (m.s.d.) of a particle undergoing forward transport (a) is compared with that of a diffusing particle (b). The data are from the trajectories of the particles shown in Fig. 1. For a particle diffusing over a period of time t , $\text{m.s.d.} = 4Dt$; for a particle being transported at constant velocity V , $\text{m.s.d.} = (Vt)^2$. For a particle which is both diffusing and being transported, $\text{m.s.d.} = 4Dt + (Vt)^2$ (ref. 21; H. Qian *et al.*, manuscript in preparation). Hence the random and directed components of motion of a particle can be extracted by fitting the measured m.s.d. to this equation. Curve *a* fits the equation $\text{m.s.d.} = 0.5314t^2 + 0.252t$ to yield $D = 6.3 \times 10^{-2} \text{ nm}^2 \text{ s}^{-1} = 6.2 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ and $V = 0.73 \mu\text{m s}^{-1}$. Curve *b* has negative curvature. This is inconsistent with directed transport and indicates that the diffusion of the particle is constrained over distances that are large compared with the particle size (H. Qian *et al.*, in preparation). The local diffusion coefficient can be obtained by fitting to a straight line, $\text{m.s.d.} = 0.1t$, to yield $D = 2.5 \times 10^{-2} \text{ nm}^2 \text{ s}^{-1} = 2.5 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ and $V = 0$. (The deviation of the m.s.d. from linearity for $t > 0.3 \text{ s}$ suggests that the constraints on diffusion become significant for $\text{m.s.d.} > 0.03 \mu\text{m}^2$.)

To test the statistical significance of the directed-motion component of curve *a*, we fit the data first to the equation $\text{m.s.d.} = 4Dt$ and then to the equation $\text{m.s.d.} = 4Dt + (Vt)^2$, using polynomial regression. The F test for improvement of fit indicated significant improvement ($P < 0.001$) when the directed motion component, $(Vt)^2$, was included. The same test when applied to curve *b* showed no improvement of fit ($P > 0.5$) when the data were fit by the second equation. Note that the values of D for the randomly moving particles range from 1×10^{-10} to $1\text{--}3 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$. The upper end of this range is rapid for diffusion of membrane proteins and approaches the rates observed for rhodopsin in rod outer segments or proteins in membrane blebs²².

Also, previous studies have concentrated on fibroblasts, which are only slowly motile. Fish epidermal keratocytes move at $30 \mu\text{m min}^{-1}$ (ref. 11) (compared with $0.5\text{--}1 \mu\text{m min}^{-1}$ for fibroblasts¹²). Hence, transport events may occur at higher frequency on the keratocytes. We have, however, observed forward transport of membrane proteins on mammalian cells as well (unpublished observations). Thus forward transport may occur generally on locomoting cells.

We considered the possibility that the observed forward particle movements are a manifestation of a lamellipodial extension mechanism. Detailed motion analyses show that forward particle motion is not temporally correlated with extension of the leading edge. As seen in Fig. 4, particles move forward even when the edge is temporarily retracting or oscillating. In stationary cells, however, no forward particle movements were observed. There-

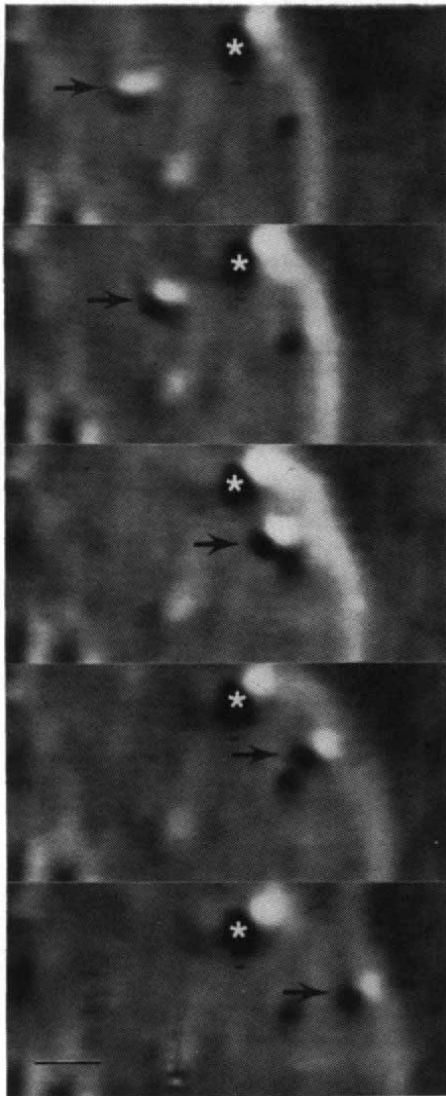


FIG. 3 Video micrographs showing the rapid forward movement of a particle to the leading edge. Frames are photographed at 1-s intervals. White asterisk in each frame, particle bound to the glass substrate and used as a position marker; black arrows, particle undergoing forward transport. Note that the leading edge extends during this sequence, but that the motion of the particle is rapid enough to overtake it.

METHODS. Microscopy was performed using a Zeiss IM-35 inverted microscope equipped with DIC optics. A Zeiss 63/1.4 planapo oil-immersion objective and an oil-immersion condenser (NA 1.4) were used. Video images were obtained using a Dage 67 camera and processed with a Hughes model 794 video digitizer. Images were then photographed from the television monitor with a 35-mm camera.

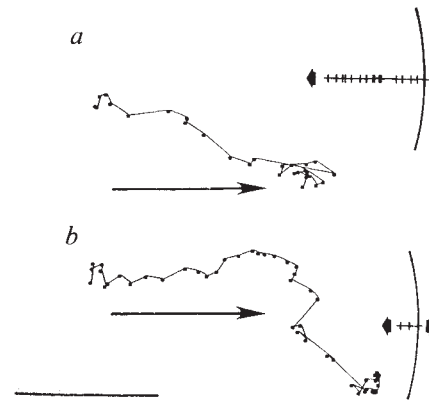


FIG. 4 Forward transport is not temporally correlated with edge extension. Positions of con A-coated gold particles are plotted along with the positions of the leading edge in cases where particles moved forwards while the leading edge moved backwards (a) or oscillated back and forth (b). Scale bar, $1 \mu\text{m}$.

fore, although this phenomenon seems to be related to locomotion, it is not directly linked to lamellipodial extension.

The mechanism of these particle movements has not yet been determined. Note, however, that although double-headed myosin II has not been detected in active regions of lamellipodia in locomoting cells, the single-headed myosin I has recently been found to be localized to the leading edge in the slime mould *Dictyostelium*¹³. This type of myosin binds both to actin filaments¹⁴ and to membrane sites¹⁵, and moves on actin filaments *in vitro*¹⁶. Thus, myosin I is likely to be the motor powering these forward movements, either by direct linkage to membrane proteins or, more likely, indirectly by binding to small actin filaments which intermittently attach to glycoproteins.

Newly synthesized viral proteins are first detected at the leading edge of motile fibroblasts¹⁷. Similarly, the low-density lipoprotein and transferrin receptors are localized to active cell protrusions of giant HeLa cells⁴. The accumulation of these proteins at the front of the cell has previously been taken as evidence for membrane insertion at the leading edge; however, the absence of significant quantities of vesicular organelles in the lamellipodium argues against that mechanism. Active forward transport of material on the cell surface has not previously been considered to participate in these processes because only rearward transport of surface particles had been observed. We suggest that this forward transport is the means by which the cell can concentrate membrane and cytoplasmic proteins at its leading edge. □

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1. Abercrombie, M., Heaysman, J. E. M. & Pegrum, S. M. *Exp. Cell Res.* **62**, 389-398 (1970).
2. Dembo, M. & Harris, A. K. *J. Cell Biol.* **91**, 528-536 (1981).
3. Bray, D. *Proc. natn. Acad. Sci. U.S.A.* **65**, 905-910 (1970).
4. Bretscher, M. S. *Science* **224**, 681-686 (1984).
5. Heath, J. P. *J. Cell Sci.* **60**, 331-354 (1983).
6. Wang, Y.-L. *J. Cell Biol.* **101**, 597-602 (1985).
7. Forscher, P. & Smith, S. J. *J. Cell Biol.* **107**, 1505-1516 (1988).
8. Fisher, G. W., Conrad, P. A., DeBiasio, R. L. & Taylor, D. L. *Cell Motil.* **11**, 235-247 (1988).
9. Gelles, J., Schnapp, B. J. & Sheetz, M. P. *Nature* **331**, 450-453 (1988).
10. Schreiner, G. & Unanue, E. R. *Adv. Immun.* **24**, 37-165 (1976).
11. Euteneuer, U. & Schliwa, M. *Nature* **310**, 58-61 (1984).
12. Harris, A. K., Stopak, D. & Wild, P. *Nature* **290**, 249-251 (1981).
13. Fukui, Y., Lynch, T. J., Brzeska, H. & Korn, E. D. *J. Cell Biol.* **107**, 471a (1988).
14. Brzeska, H., Lynch, T. J., & Korn, E. D. *J. Biol. Chem.* **263**, 427-435 (1988).
15. Adams, R. J. & Pollard, T. D. *Nature* **322**, 754-756 (1988).
16. Albanesi, J. P. et al. *J. Biol. Chem.* **260**, 8649-8652 (1985).
17. Bergmann, J. E., Kupfer, A. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1367-1371 (1983).
18. Berg, H. C. *Random Walks in Biology* (Princeton University Press, 1983).
19. Kolega, J. *J. Cell Biol.* **102**, 1400-1411 (1986).
20. Cooper, M. S. & Schliwa, M. *J. Cell Biol.* **102**, 1384-1399 (1986).
21. Sheetz, M. et al. *Nature* **340**, 284-288 (1989).
22. Jacobson, K., Ishihara, A. & Inman, R. A. *Rev. Physiol.* **49**, 163-175 (1987).

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Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast

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ALTHOUGH single heterozygous markers in yeast usually segregate during meiosis in a 2:2 ratio, aberrant 3:1 segregations occur quite frequently as a result of gene-conversion events¹. A second type of aberrant segregation, post-meiotic segregation, results from the segregation of two genotypes from a single haploid spore; in yeast such events are detected as sectorized spore colonies² and usually occur rarely¹. Post-meiotic segregation is thought to result from the replication of heteroduplex DNA formed during meiotic recombination. We report here that if the heteroduplex includes a palindromic insertion sequence, a high frequency of post-meiotic segregation results. This suggests that palindromic insertions are poorly repaired, which may be the result of hairpin-loop formation that affects the efficiency of repair of heteroduplex DNA.

During meiosis, two heterozygous alleles, *A* and *a*, can replicate and segregate to give a variety of combinations of *A* and *a* in the progeny. Following conventions established for fungi containing 8-spored asci, the segregation patterns can be classified as: normal 4*A*:4*a* (2*A*:2*a* spore colonies), 6*A*:2*a* and 2*A*:6*a* (3*A*:1*a* and 1*A*:3*a* spore colonies; gene conversion), 5*A*:3*a* and 3*A*:5*a* (2*A*:1*a*:1*A*/*a* spore colonies or 1*A*:2*a*:1*A*/*a* spore colonies; one post-meiotic segregation event per tetrad) and aberrant 4*A*:4*a* (1*A*:1*a*:2*A*/*a* spore colonies; two post-meiotic segregation events per tetrad). Both conversion and post-meiotic segregation events are often associated with crossing-over of flanking sequences¹.

Two models of recombination have been invoked to explain these segregation patterns (Fig. 1). In the Meselson-Radding model³ (Fig. 1*a*), both conversion and post-meiotic events reflect asymmetric heteroduplex formation, with repair of the resulting

mismatch leading to conversion (or restoration of the parental genotype) and failure to repair leading to post-meiotic segregation. In this model, heteroduplex formation involves a single chromatid and only one post-meiotic segregation event per tetrad is expected, whereas an earlier model⁴, in which heteroduplexes formed symmetrically on both chromatids, predicted that tetrads with two post-meiotic segregation events would be common. In the double-strand break model⁵ (Fig. 1*b*), most conversion events reflect repair of a double-strand gap and post-meiotic segregation events are the result of failure to repair the mismatch in the heteroduplex flanking the gap as they are in the other models.

To test these models, we analysed the segregation of various types of mutational changes at the *HIS4* locus in yeast. The first mutant allele examined (*his4-Sal*) was constructed by 'filling-in' a *SalI* restriction site near the 5' end of the gene (Fig. 2*a*) to create a 4-base-pair (bp) frame-shift mutation^{6,7}. The mutant sequence was substituted for the wild-type sequence in one of the two homologous chromosomes of the diploid strain MW103. When the strain was sporulated at 18 °C and dissected, 28% of the tetrads showed gene conversion (an extraordinarily high rate) and 3% showed 8:0 or 0:8 segregation, probably representing double meiotic conversion events. In this strain, loci other than *HIS4* converted at normal rates (Table 1) and no post-meiotic segregation events were observed for *his4-Sal* (Table 1), which is consistent with previous studies^{6,7}.

To examine the effects of longer insertion mutations on recombination at a site with a high rate of gene conversion, we constructed a mutant allele (*his4-lop*) that had a 26-bp oligonucleotide containing the *lexA* operator⁸ (provided by R. Brent) inserted into the *HIS4* gene at the *SalI* site (Fig. 2). This sequence is a perfect palindrome. A yeast strain (DNY11), heterozygous for this insertion (and isogenic with MW103), was constructed and analysed. Although the rate of conversion was less for this allele than for *his4-Sal*, the rate of post-meiotic segregation was dramatically higher (Table 1).

These results are most simply explained by the Meselson-Radding model. The frequencies of total aberrant segregations are similar for *his4-Sal* and *his4-lop*. The concerted changes in the levels of gene conversion and post-meiotic segregation in the two strains are consistent with the hypothesis that conversion

TABLE 1 Number of tetrads with aberrant meiotic segregation of different mutant *his4* and *leu2* alleles

Strain*	Mutant allele	5:3	3:5	7:1	1:7	Aberrant	4:4	6:2	2:6	8:0	0:8	Others†	Total no. tetrads	Aberrant segregation (% of total)	PMS (% of total ab.)
MW103	<i>his4-Sal</i>	0	0	0	0	0	31	50	2	6	0	0	291	30.6	0
DNY11	<i>his4-lop</i>	36	32	3	1	2	23	6	1	0	5	0	312	34.9	72.47
DNY18	<i>his4-B2</i>	49	41	2	1	8	36	20	4	2	2	0	382	43.2	62.4
DNY27	<i>his4-lopd</i>	2	4	0	0	0	41	39	0	1	0	0	440	19.7	6.8
DNY26	<i>his4-lopc</i>	40	37	2	0	8	18	15	1	0	2	0	394	31.2	79.3
All above	<i>leu2-Bs</i>	0	0	0	0	0	32	33	1	0	0	0	1,819	3.6	0
DNY23	<i>leu2-lop</i>	3	5	0	2	0	3	22	0	1	0	0	430	8.37	30.5

Number of tetrads with aberrant meiotic segregation of different mutant *his4* and *leu2* alleles. Diploid strains heterozygous for various *his4* and *leu2* mutations were induced to undergo meiosis and the resulting tetrads were dissected. The normal meiotic segregation is 4⁺:4⁻, with no post-meiotic segregation (PMS) events. The frequencies of gene conversion for other markers in the strains (1,958 tetrads were analysed) were: 9.4% (*arg4-17*), 1.4% (*trp 1-1*) and 3.1% (*tyr7-1*).

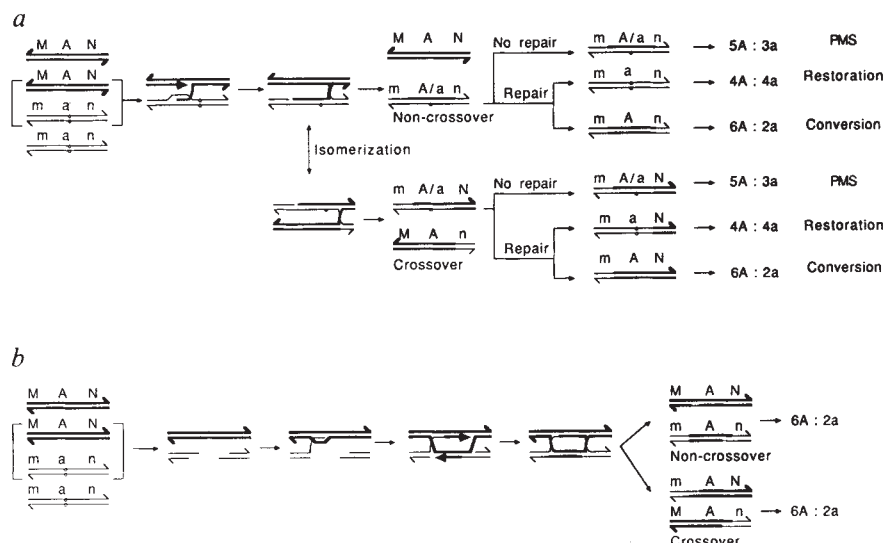
* All diploid strains were constructed by crossing one haploid in the AS4 background (*α-trp1-1 arg4-17 tyr7-1 ura3 ade6*) to a haploid with the AS13 background (*a leu2-Bs ura3-52 ade6*); these strains are closely related to those used by Symington and Petes⁵. In all cases, except DNY23, the altered *his4* genes on plasmids were used to replace the wild-type sequences in AS13 (two-step transplacement¹⁸) and the resulting transformants were mated to AS4 to make the diploids. The plasmids used for each construction (described in Fig. 2; all contain *URA3*) were: MW103 (pC1G17; provided by L. Symington), yeast strain constructed by M. White; DNY11 (pDN4); DNY18 (p627); DNY27 (pDN12); and, DNY26 (pDN13). DNY23 was constructed by replacing *leu2-Bs* with *leu2-lop* using pDN11, in LS3-17c (a *LEU2* strain isogenic with AS13; ref. 6) and mating the resulting transformant to PD7 (constructed by P. Detloff). PD7 is isogenic with AS4, except *his4-519* sequences replace *his4-Sal*.

† Other classes include tetrads with two post-meiotic segregation events and two spores with the same genotype (aberrant 6:2 and 2:6) and tetrads with three post-meiotic segregation events (deviant 5:3 and 3:5). These classes can be explained as follows: aberrant 4:4 (rare symmetric heteroduplexes or two independent asymmetric heteroduplexes), aberrant 6:2 and 2:6 (two unrepaired asymmetric heteroduplexes) and deviant 5:3 and 3:5 (either one symmetric and one asymmetric heteroduplexes or two cycles of asymmetric heteroduplex formation involving the same chromosomes). The specific classes found in each strain were: DNY11 (two aberrant 6:2, one deviant 5:3 and two deviant 3:5), DNY18 (one aberrant 6:2 and one aberrant 2:6) and DNY26 (two aberrant 2:6).

FIG. 1 Models of recombination. The four pairs of lines indicate the four chromatids present after DNA replication; the thick lines represent one homologue and the thin lines the other. The two DNA molecules involved in the recombination event are bracketed. Gene *A* is located at the breakpoint of the recombination event and genes *M* and *N* are flanking markers. Wild-type alleles are capitalized. The mutant substitution in *a* is indicated by the small circles on the DNA strands.

a, Meselson-Radding model³. One of the DNA molecules is nicked; the resulting free end serves as a primer for DNA synthesis, displacing a single strand. This strand invades the homologous chromosome, resulting in a D-loop. The displaced strand is degraded. The *A/a* mismatch at this stage is an asymmetric heteroduplex. This intermediate can undergo a rotation of two chromatid ends relative to the other two (isomerization) in order to arrange the flanking markers in the recombinant configuration. The structure is resolved by cutting the crossing strands. Repair of the *A/a* mismatch results in gene conversion or restoration; failure to repair a mismatch will produce post-meiotic segregation.

b, Double-strand break model⁵: a double-strand break is enlarged into a gap, flanked by broken molecules with 3' overhangs. One of these molecules invades the other homologue and repair synthesis results in D-loop formation. The displaced strand pairs with the other broken end and a second round of repair synthesis occurs. Resolution of the double-Holliday structure



occurs by cleaving either the inner or outer strands of each junction. Cleavage of both junctions in the same sense results in flanking markers in the parental configuration; cleavage in the opposite sense results in cross-over tetrads. Conversion is the result of gap repair or repair of a mismatch in the small region of heteroduplex flanking the gap. Post-meiotic segregation (PMS) is the result of failure to repair the mismatch in the heteroduplex.

and post-meiotic segregation represent two alternative fates of mismatches in a heteroduplex. Thus, the high rate of conversion and low rate of post-meiotic segregation for *his4-Sal* reflects a high rate of heteroduplex formation at this locus and efficient repair of the resulting mismatch. Although heteroduplex formation is also efficient with the *his4-lop* allele, the resulting mismatch is inefficiently repaired, resulting in a lower conversion rate and a greatly increased rate of post-meiotic segregation. The conclusion that most of the conversion events at this locus are the result of mismatch repair is consistent with earlier studies of high post-meiotic segregation alleles at the yeast *ARG4* locus⁹. Additionally, mutants have been isolated¹⁰ that give rise to higher levels of post-meiotic segregation and lower levels of gene conversion for certain mutant alleles, as we would expect if conversion is the result of mismatch repair.

In a heteroduplex formed between a DNA strand containing an insertion and a strand without insertion, the insertion is expected to form a single-stranded loop. As the *his4-lop* insertion is palindromic, its predicted configuration is a hairpin, formed by intra-strand pairing, rather than a loop (Fig. 3). Structural constraints¹¹ suggest that four unpaired bases should occur at the tip of the hairpin; our results indicate that such structures are poorly recognized by the cellular mismatch repair systems. To demonstrate that the observed high rate of post-meiotic segregation was a consequence of the structure rather than the sequence of the insertion, we examined the properties of several other insertions.

One mutant allele (*his4-lopd*) contained an oligonucleotide with four single bp changes that were predicted to interfere with the stability of the hairpin (Fig. 3). The yeast strain containing these changes (DNY27) had an increased conversion rate and decreased post-meiotic segregation rate relative to DNY11 (Table 1), indicating that high post-meiotic segregation is not a feature of all 26-bp insertions at this site. We next examined *his4-lopc* which had an oligonucleotide with base-pair changes that compensated for the mismatches of *his4-lopd* (Fig. 3). The yeast strain with this mutant allele had high levels of post-meiotic segregation and a low rate of conversion (Table 1). As the sequence of this insertion is quite different from the original oligonucleotide, the most probable explanation of our results is that secondary structure determines the level of post-meiotic segregation.

We also studied conversion and post-meiotic segregation in a strain containing a mutant *his4* allele resulting from insertion of a palindromic oligonucleotide that was unrelated in sequence to the *lexA* operator¹². This mutant allele (*his4-B2*, provided by T. Donahue) contained a 12-bp G+C-rich palindrome (Fig. 3) inserted in the leader region of *HIS4* (Fig. 2a). The strain with this insertion (DNY18) gave rise to a high level of post-meiotic segregation.

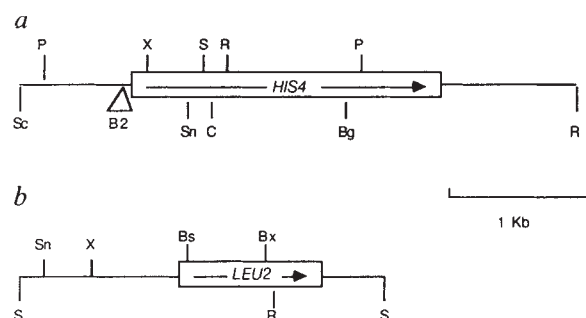


FIG. 2 Partial restriction maps of *HIS4* (a) and *LEU2* (b) regions and construction of plasmids with palindromic insertions. The boxed region indicates the coding region of the gene. Abbreviations: B2, insertion site of B2 oligonucleotide¹²; Bg, *Bgl*III; Bs, *Bst*EII; Bx, *Bst*XI; C, *Cl*AI; P, *Pvu*II; R, *Eco*RI; S, *Sal*I; Sn, *Sna*BI; X, *Xho*I.

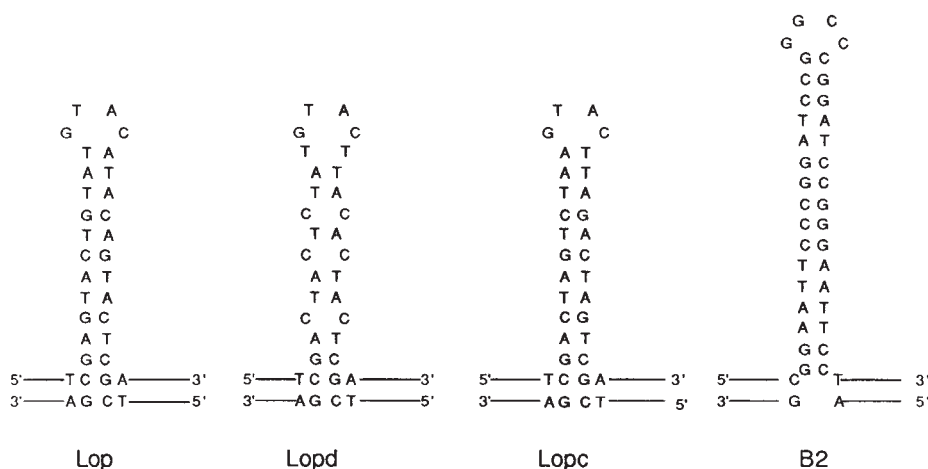
METHODS. All plasmid constructions were made by standard procedures¹⁹. *his4-Sal*, plasmid pC1G (ref. 20) was partially digested with *Sal*I and the resulting recessed ends were filled-in by DNA polymerase and ligated together (pC1G17, provided by L. Symington); *his4-lop*, the 26-bp oligonucleotide with the *lexA* operator (see Fig. 3) and *Sal*I-compatible ends was annealed and inserted into *Sal*I site of pMK45 (*Sac*I-*Spe*I *HIS4* fragment in *Sma*I site of pUC19, provided by M. Kupiec, Tel Aviv Univ.) to make pDN3; a *URA3* gene was then inserted into the *Sal*I site of pDN3 to make pDN13; *his4-lopd*, two complementary oligonucleotides (see Fig. 3) were annealed and inserted into *Sal*I site of pDN9 (*Xho*I-*Bgl*II *HIS4* fragment in *Sal*I-*Bam*HI treated Ylp5) to make pDN12; *his4-lopc*, the *lop*c oligonucleotide (see Fig. 3) was annealed and inserted into the *Sal*I site of pDN9 to make pDN13; p627 (containing *his4-B2*), as described in Cigan *et al.*¹²; pLS34 (with *leu2-Bs*), as described by Symington and Petes⁶; *leu2-lop*, an oligonucleotide with the *LexA* operator sequences, but with a 5' end compatible with *Bst*EII rather than *Sal*I, was annealed and inserted into the *Bst*EII site of the *LEU2* gene in pG4B (ref. 20) to make pDN11.

FIG. 3 Hairpins formed in heteroduplexes involving one wild-type strand and one strand with a palindromic insertion. Shown in this diagram is the intra-strand pairing expected for heterozygous palindromic insertions during heteroduplex formation *in vivo*. The bottom strand contains wild-type information. We show four bases at the tip of the hairpin as unpaired.¹¹

METHODS. The oligonucleotides shown, except for B2, have the sequence T-C-G-A at the 5' end. As *lop*, *lop*c and B2 sequences are perfect palindromes, bimolecular annealing of such sequences should result in a double-stranded structure, as shown below for *lop*:

```
5' TCGAGTACTGTATGTACATACAGTAC 3'
3' CATGACGTACATGTATGTCATGAGCT 5'
```

As the *lop*d oligonucleotide is not a perfect palindrome, a similar structure was formed by annealing two complementary sequences. The double-stranded oligonucleotides for *lop*, *lop*c and *lop*d were then inserted into the *Sall* restriction site within the *HIS4* gene (see Fig. 2).



To determine whether the effect of palindromic insertions on post-meiotic segregation is unique to the *HIS4* locus, we inserted the *lexA* operator into the *LEU2* gene (Fig. 2b). The frequency of post-meiotic segregation was high (Table 1) relative to that of the most recently described mutant alleles in yeast, but not as high as that observed at *HIS4*, indicating that repair of the mismatch may be affected by neighbouring DNA sequences or closely linked heterozygosity.

Several conclusions can be drawn from this study. First, in at least some situations, secondary structure in DNA is biologically important. Less direct evidence supporting this conclusion has also been provided by studies of certain deletion events in *Escherichia coli* and yeast¹³⁻¹⁶. It is important to stress that, in our experiments, intra-strand pairing is facilitated by heteroduplex formation between a strand with an insertion and a wild-type strand. We do not know whether intrastrand pairing (resulting in cruciform formation) also occurs in the absence of heteroduplex formation. Second, our results suggest that the yeast mismatch repair system is insensitive to several different types of alterations. Previously, *ade8-18*, a 38-bp deletion,⁹ and *arg4-16*, a G→C transversion⁹ were the only mutant alleles in yeast known to have high rates of post-meiotic segregation; the 38-bp insertion that is present in wild-type strains relative to the *ade8-18* allele is not obviously palindromic. Most large (>100-bp) insertions or deletions in yeast have normal rates of gene conversion¹⁷ but do not give rise to high rates of post-meiotic segregation. Third, strains with high levels of post-meiotic segregation, usually exhibit tetrads that have a single post-meiotic segregation event (5:3 or 3:5), rather than two (aberrant 4:4) or three (see Table 1). The frequency of tetrads showing double post-meiotic segregation events is low enough that most of these probably reflect independent formation of

two asymmetric heteroduplexes rather than concerted formation of a symmetric heteroduplex. This result confirms earlier data¹ which indicated that heteroduplex formation in yeast is usually asymmetric rather than symmetric.

Our results suggest that most conversion events are the result of mismatch repair in a heteroduplex rather than gap repair. To explain the effects of the palindromic insertions and other high post-meiotic segregation alleles by the double-strand break model, Szostak *et al.*⁵ suggested that these alleles cause a pause site for the exonuclease that generates the gap; it would then be likely that sequences near the pause site would be included in the heteroduplex. Because this effect would be seen only for the chromosome with the high post-meiotic segregation allele, one would expect that this chromosome would act as a donor in segregation events more frequently than the wild-type donor. But as we find that 5:3 and 3:5 tetrads are equally frequent, our results are not consistent with this mechanism. They do not exclude the possibility that recombination is initiated by a double-strand break whereas conversion events are the result of mismatch repair.

The methods described above should be useful in constructing high post-meiotic segregation alleles at essentially any position in the genome. In addition, these alleles may allow the physical detection of heteroduplexes as DNA fragments with heterozygous hairpins would be expected to migrate anomalously during gel electrophoresis. The *HIS4* locus is likely to be a particularly good gene for such studies as 25% of unselected tetrads of strains DNY11, DNY18 and DNY26 had a post-meiotic segregation event at this site. This level of heteroduplex formation is considerably higher than that observed previously for any yeast gene. □

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1. Fogel, S., Mortimer, R. K. & Lusnak, K. in *The Molecular Biology of the Yeast Saccharomyces* (eds Strathern, J., Jones, E. W. & Broach, J.) Vol. 1, 289-339 (Cold Spring Harbor Laboratory, New York, 1981).
2. Esposito, M. S. *Molec. gen. Genet.* **111**, 297-299 (1971).
3. Meselson, M. & Radding, C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 358-361 (1975).
4. Holiday, R. *Genet. Res.* **5**, 282-304 (1984).
5. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. *Cell* **33**, 25-35 (1983).
6. Symington, L. S. & Petes, T. D. *Molec. cell. Biol.* **8**, 595-604 (1988).
7. Borts, R. H. & Haber, J. E. *Science* **237**, 1459-1465 (1987).
8. Brent, R. & Ptashne, M. *Nature* **312**, 612-615 (1984).
9. White, J. H., Lusnak, K. & Fogel, S. *Nature* **315**, 350-352 (1985).
10. Williamson, M. S., Game, J. C. & Fogel, S. *Genetics* **110**, 609-646 (1985).
11. Gough, G. W., Sullivan, K. M. & Lilley, D. M. *J. EMBO J.* **5**, 191-196 (1986).
12. Cigan, A. M., Pabich, E. K. & Donahue, T. F. *Molec. cell. Biol.* **8**, 2964-2975 (1988).

13. Dasgupta, U., Weston-Hafer, K. & Berg, D. E. *Genetics* **115**, 41-49 (1987).
14. Glidman, B. W. & Ripley, L. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 512-516 (1984).
15. McClain, W. H. *J. molec. Biol.* **204**, 27-40 (1988).
16. Warren, G. J. & Green, R. L. *J. Bacteriol.* **161**, 1103-1111 (1985).
17. Fink, G. J. & Styles, C. A. *Genetics* **77**, 231-244 (1974).
18. Scherer, S. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4951-4955 (1979).
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
20. Newlon, C. *et al.* *UCLA Symp. molec. cell. Biol.* **33**, 211-223 (1986).

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Measurement of anomalously high hydration of (dA)_n · (dT)_n double helices in dilute solution

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DIFFERENT DNA sequences have different physical properties, which seem to be important for their biological function¹⁻³. In particular, (dA)_n · (dT)_n has many unusual features^{1,4}, which include resistance to conformational changes in a variable chemical environment⁵, an unusual thermodynamics of interaction with ligands⁶, and the inability to reassociate into nucleosomes⁷. Short A · T base-pair runs also play a critical role in DNA bending⁸. It is believed that hydration of DNA is an important factor in determining the physical chemical and biological properties of different regions of DNA¹. Until now, however, it has not been possible to study the details of the hydration of DNA in dilute solution with sufficient sensitivity and precision. Moreover, it was not known if different base sequences differ in the extent of their hydration. Indirect evidence that (dA)_n · (dT)_n can be hydrated to a greater extent than other DNA sequences may be inferred from a recent study of the binding of drugs to polynucleotides⁶. Here we used a novel high-precision technique measuring ultrasonic velocity⁹ to obtain direct estimates of the extent of hydration of various oligo- and polynucleotides in dilute solution. We report that different DNA sequences differ in their hydration, and that (dA)_n · (dT)_n in particular has an anomalously high level of hydration.

Ultrasound velocity and density measurements have been used widely to estimate the hydration of ions¹⁰, simple molecules, and polymers in dilute solution¹¹. The development of a more sensitive small-scale version of this technique enabled us to investigate the details of the hydration of DNA. This resonance technique measures relative changes in ultrasonic velocity, at frequencies of about 7 MHz, in small volumes of DNA solutions (0.8 ml) at concentrations of less than 1 mg ml⁻¹. The technique has a precision of about 10⁻⁵%^{9,12}. These velocity values, together with those of the density of the solution (measured using an Anton Paar DMA 602 densitometer), were used to find the apparent molar compressibility (Φ_{ks}) and the apparent molar volume (Φ_v) of the solutions¹¹. These parameters can be used to estimate the hydration of molecules in dilute solution^{12,13}:

$$\Phi_v = \bar{V}_M + \Delta V_h \quad (1)$$

$$\Phi_{ks} = \bar{K}_M + \Delta K_h \quad (2)$$

where $\bar{V}_M$ is the volume of the solute molecule which is inaccessible to the solvent, $\bar{K}_M$ is the compressibility of this solute volume, and ΔV_h and ΔK_h respectively describe the changes in the volume and compressibility of water surrounding the DNA as a result of hydration. For the DNA double helix, $\bar{K}_M$ is small and may be neglected, especially in the case where we compared helices having different base sequences¹⁴.

The apparent molar compressibility (Φ_{ks}), which is therefore equal to ΔK_h , is sensitive to changes in the degree of hydration. In general, the less the hydration of a molecule, the higher its Φ_{ks} value. Ions have large negative values of Φ_{ks} because the compressibility of the water at their surface is decreased

as a result of electrostatic effects¹⁰. For example, Na₂HPO₄ is highly hydrated and has a Φ_{ks} value of $-170 \times 10^{-4} \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1}$. Sugars such as ribose, which can only form hydrogen bonds, have a lower hydration and larger values of Φ_{ks} of about $-12 \times 10^{-4} \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1}$ (ref. 15). Molecules such as purine, which are to a large extent hydrophobic, have values of Φ_{ks} close to zero¹². A similar inverse relationship exists between the extent of hydration and the relative magnitudes of the parameter $\Phi_v - \bar{V}_M$ ¹².

Oligonucleotides were chemically synthesized as described¹⁶; their sequences are shown in Fig. 1. They were dissolved in CsCl (0.2 mol l⁻¹) (which, because of its low contribution to ultrasound velocity, was chosen in preference to NaCl), and the measurements were made at 1 °C to maximize the stability of the helices. These conditions ensured that all oligonucleotides formed B-type double helices, as determined from their ultraviolet melting curves and circular dichroism spectra. As an additional test for B-type conformation, the least stable oligonucleotide (number 4 in Fig. 1) was titrated within the ultrasonic resonance cell with different concentrations of CsCl. No change was detected in acoustical parameters for this oligonucleotide over the range 0.1 to 0.8 mol l⁻¹. As the concentration of CsCl was decreased below 0.1 mol l⁻¹, however, there was a pronounced increase in hydration, indicating dissociation of the helices into single strands.

Figure 2 presents the values of Φ_{ks} and $\Phi_v - \bar{V}_M$ for the sodium salts of six oligonucleotide and three polynucleotide duplexes. Generally, for each class of molecules, an increase in the A + T content gave a decrease in the values of both Φ_{ks} and $\Phi_v - \bar{V}_M$, indicating a rise in the extent of hydration. This result is in agreement with data obtained using buoyant density measurements¹⁹ and calorimetric²⁰ techniques.

The data obtained with 100% A + T sequences also demonstrates that the hydration of nucleic acids in dilute solution depends on their base sequences as well as their base composition. This is shown in Fig. 2 by the abnormally low values of

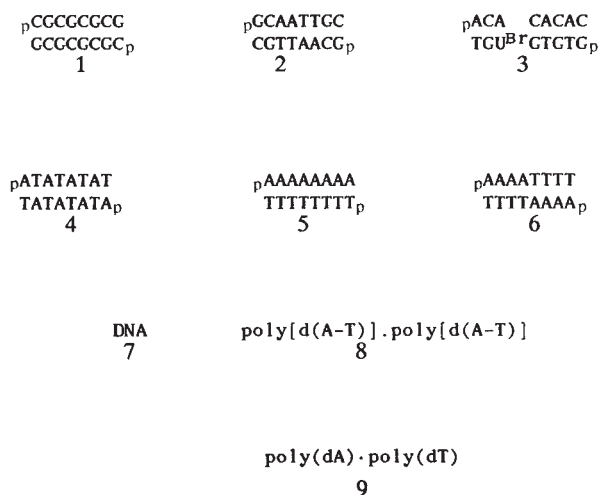


FIG. 1 The base-pair sequences of the deoxyoligonucleotides. In sequence no. 3, U^{Br} stands for 5-bromodeoxyuracil. The method of synthesis is described in ref. 16, and their sequences were confirmed by the Maxam-Gilbert method¹⁷. They were purified by ion-exchange chromatography in potassium phosphate buffer (0 to 0.4 mol l⁻¹), pH 7.5, containing urea (7 mol l⁻¹), followed by double counter-current chromatography (on Lichrosorb-5RP-18 sorbent) in acetonitrile (0-20%) containing NaClO₄ (0.05 mol l⁻¹). Excess salt was removed by double elution through a Sephadex-G10 column using double glass-distilled water, pH 6.5-7. The deoxyoligonucleotide solutions were lyophilized, and re-dissolved in CsCl (0.2 mol l⁻¹) buffered at pH 7.8 with HEPES buffer (2 mmol l⁻¹). The concentrations of the oligonucleotides were determined from their extinction coefficients after digestion with snake-venom phosphodiesterase.

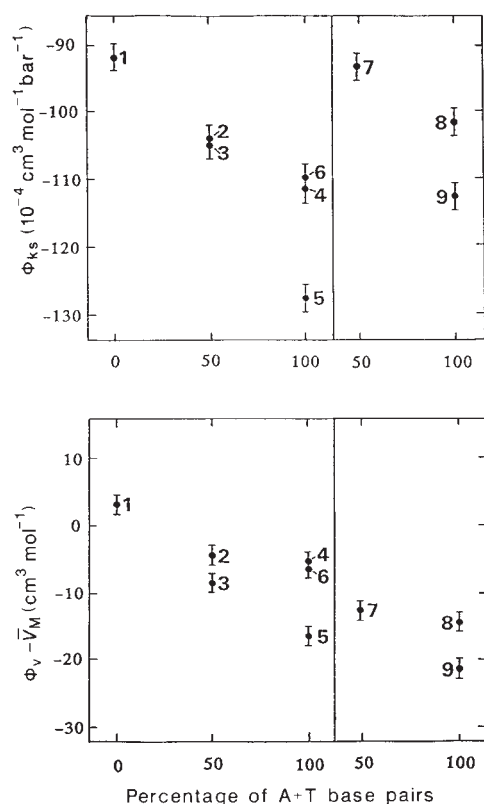
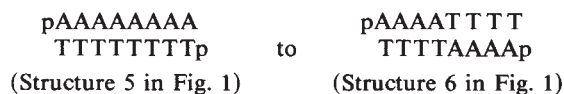


FIG. 2 The measured values of Φ_{KS} and $\Phi_V - \bar{V}_M$ of the sodium salts of oligo- and polynucleotides as a function of the percentage of A+T base pairs. The measurements were made at 1 °C in CsCl (0.2 mol l⁻¹) HEPES buffer (2 mmol l⁻¹), pH 7.8, at concentrations of ~1 mg ml⁻¹. Numbers 1 to 6, deoxyoligonucleotide sequences described in Fig. 1; 7, salmon-sperm DNA; 8, the alternating poly[d(A-T)] · poly[d(A-T)]; and 9, the homopolymer poly(dA) · poly(dT). Note that generally, the lower the values of both Φ_{KS} and $\Phi_V - \bar{V}_M$, the higher the degree of hydration. All values are calculated per mole of nucleotide. The details of the measurements and preparative procedure are described elsewhere^{12,14}. The values of $\bar{V}_M$ were taken from ref. 18 and the intrinsic volume of sodium ions was not included in the calculations.

both Φ_{KS} and $\Phi_V - \bar{V}_M$ obtained with the oligo(dA) · oligo(dT) and the poly(dA) · poly(dT) duplexes. An important observation is that changing the sequence of the molecule from



resulted in a large decrease in its hydration. These structures differ in only one base-pair stacking contact in the middle of the helix.

The evidence presented here indicates that (dA)_n · (dT)_n tracts have an anomalously high hydration in dilute solution. An enhanced hydration has been proposed to be one of major determinants of the anomalous properties of (dA)_n · (dT)_n.^{21,22} □

14. Buckin, V. A., Kankiya, B. I., Sarvazyan, A. P. & Uedaira, H. *Nucleic Acids Res.*, in the press.
15. Lo Surdo, A., Shin, Ch., Millero, F. J. *J. chem. Engng. Data* **23**, 187-201 (1978).
16. Ivanova, E. M., Kutiavin, I. V., Pietnev, A. G. & Shamanin, V. A. *Bioorg. Chem.* **8**, 1501-1515 (1982).
17. Maxam, A. G. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1986).
18. Pavlov, M. Ya. & Fedorov, B. A. *Biofizika* **28**, 931-936 (1983).
19. Tunis, M. J. B., Hearst, J. E. *Biopolymers* **6**, 1325-1344 (1968).
20. Mrevlishvili, G. M. *Dokl. Akad. Nauk. SSSR* **260**, 761-764 (1981).
21. Chuprina, V. P. *FEBS Lett.* **186**, 98-102 (1985).
22. Chuprina, V. P. *FEBS Lett.* **195**, 363 (1986).

ERRATA

Creation of the Uranus rings and dust bands

L. W. Esposito & Joshua E. Colwell

Nature **339**, 605-607 (1989)

AN error was introduced during the processing of this letter. The two sentences beginning seven lines below equation 3 on page 606 should read: 'The satellites 1986U1-9 range from 20 to 40 km in radius. We consider this a confirmation, but it shows no contradiction between the model disruption rate (calculated from the crater⁵) and the observed moons near the rings.' □

Triple-strand formation in the homopurine:homopyrimidine DNA oligonucleotides d(G-A)₄ and d(T-C)₄

Ponni Rajagopal & Juli Feigon

Nature **339**, 637-640 (1989)

IN Fig. 2a of this letter, the A · T base pairs are mislabelled as G · T. In Fig. 2b the indication of Hoogsteen base pairs is omitted, but these are correctly labelled in Fig. 3. Also in Fig. 2b, the 'H-bonded aminos' label should read 'C⁺ aminos'. □

Inhibition of exocytosis by intracellularly applied antibodies against a chromaffin granule-binding protein

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Nature **339**, 709-712 (1989)

IN this letter in the 29 June issue, the affiliations of the authors were unclear in the printed version. The correct details appear above. □

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1. Saenger, W. *Principles of Nucleic Acid Structure* (Springer, New York, 1984).
2. Berowicz, J. A., Zhang, L., Sasse-Dwight, S. & Gralla, J. D. *J. molec. Biol.* **196**, 101-111 (1987).
3. Anderson, J. E., Ptashne, M. & Harrison, S. C. *Nature* **326**, 846-852 (1987).
4. Nelson, H. C. M., Finch, J. T., Luisi, B. F. & Klug, A. *Nature* **330**, 221-226 (1987).
5. Leslie, A. G. W., Arnott, S., Chandrasekaran, R. & Ratliff, R. L. *J. molec. Biol.* **143**, 49-72 (1980).
6. Breslawer, K. J. et al. *Proc. Natn. Acad. Sci. U.S.A.* **84**, 8922-8926 (1987).
7. Kunkel, G. R. & Martinson, N. G. *Nucleic Acids Res.* **9**, 6869-6888 (1981).
8. Wu, H. M. & Crothers, D. M. *Nature* **308**, 509-513 (1984).
9. Sarvazyan, A. P. *Ultrasonics* **20**, 151-154 (1982).
10. Conway, B. E. *Ionic Hydration in Chemistry* (Elsevier, Amsterdam, 1981).
11. Roy-Chowdhury, P. *Macromolec. chem. Phys.* **C27**(2), 219-252 (1987).
12. Buckin, V. A. *Biophys. Chem.* **29**, 283-292 (1988).
13. Shio, H., Ogawa, T. & Yoshihashi, H. *J. Am. chem. Soc.* **77**, 4980-4982 (1955).

Retro-secretion of recombinant proteins

J.W. Wills

The retroviral Gag protein can be used to package recombinant proteins produced by mammalian cell cultures. Protein products can then be isolated easily from the recombinant virions.

RETROVIRUSES have provided the world with some of its most challenging and frightening diseases — cancer and AIDS. But ironically, retroviruses have also provided investigators with some very important tools for understanding the molecular basis of human diseases. Reverse transcriptase, for example, has enabled the synthesis of cDNAs from eukaryotic mRNAs, greatly simplifying the analysis of the corresponding genes and protein products. Retrovirus-based expression vectors also now offer realistic hope for future gene therapies against hereditary diseases. Retro-secretion — the packaging of foreign proteins into retroviruses for concentration and purification from cell cultures — is yet another emerging technology derived from the study of retroviruses.

Proteins secreted from eukaryotic cells are generally difficult to purify from the growth medium because of the high protein content of the serum usually required for their growth. Likewise, non-secreted proteins are difficult to purify because of the total intracellular protein mass. In contrast, viral structural proteins are generally quite easy to obtain simply because they are sequestered from other intracellular proteins and packaged into particles (virions) that can be readily collected and separated from non-viral proteins by centrifugation. The retro-secretion technique enables proteins of interest to be packaged into retrovirions.

The Gag machine

Retroviruses have many features that make them particularly well suited for the packaging of foreign proteins. Unlike baculoviruses and adenoviruses, most retroviruses do not kill their host cells, allowing them to produce virions for extended periods of time¹. Retrovirions also assemble by budding from the plasma membrane², so the particles stably accumulate in the growth medium. Most importantly, however, only a single retroviral protein, the *gag* gene product, is required to drive the budding process. The viral glycoproteins (*env* products), the reverse transcriptase and accessory proteins (*pol* products), and the viral genome are all dispensable. Indeed, only a portion of the Gag protein is required for particle formation^{3,4}. Because of these properties, the Gag protein has been termed a "particle-making machine"².

Two distinct strategies for the packaging of non-viral proteins into retroviral

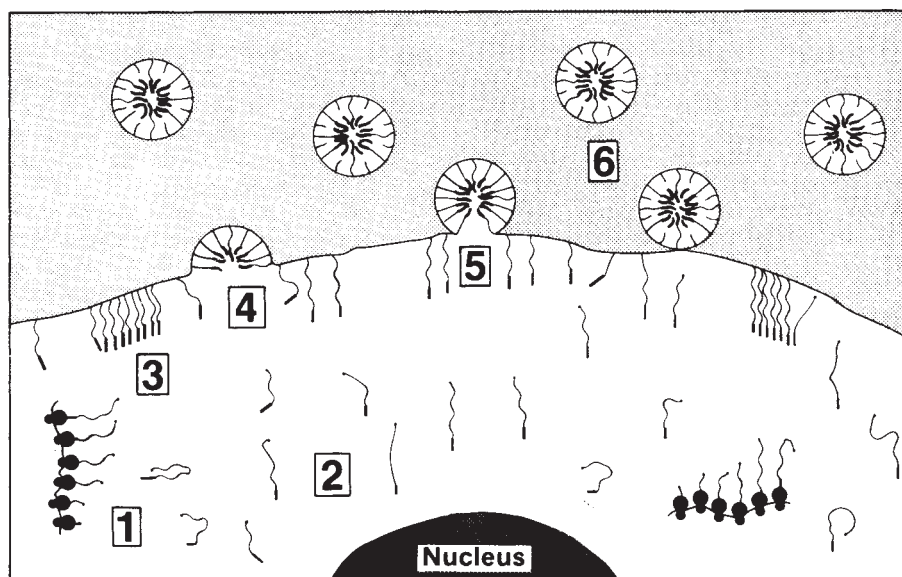


Fig. 1 The retro-secretion process (see text for details).

particles can be imagined. One would be to attach the polypeptides of interest directly to the Gag protein, allowing them to tag along as the particles are assembled. The other strategy would be to place the necessary packaging signals on glycosylated proteins of interest so that they would be incorporated in the envelope of the budding particles when the Gag protein is expressed.

Internal packaging

The molecular events involved in the retro-secretion of proteins within retroviruses are illustrated in Fig. 1. Briefly, recombinant DNA manipulations are used to link the *gag* coding sequence to that for the protein of interest. When the resulting hybrid gene is expressed, fusion proteins are synthesized on free ribosomes (step 1) and are released into the cytoplasm (step 2). The proteins contain a portion of the Gag protein (thin, squiggly lines) and the protein of interest (thick ends on lines). The Gag portion directs the protein to the plasma membrane (step 3), where high concentrations accumulate in a highly ordered manner. Interactions between the Gag portions initiate the budding process (step 4), during which the fusion proteins become enclosed in membrane and excluded from other proteins in the cytoplasm (step 5). The particles are then released into the growth medium (step 6) and can be purified by simple centrifugation. Because only a portion of the Gag protein is used in this process, the particles produced are not infectious and

are safe to handle. A somewhat related approach has been developed in yeast using the retrotransposon, Ty, but the resulting particles are not released from the yeast cells⁵.

The feasibility of using chimaeric Gag proteins to retro-secrete foreign polypeptides has recently been demonstrated⁴ using the *gag* gene from Rous sarcoma virus (RSV), an avian retrovirus. RSV is a good choice for this technology because it is one of the best understood retroviruses and has never been associated with human disease. Moreover, mammalian cells contain no RSV-related endogenous sequences which might be activated by recombination to produce infectious virions.

But RSV does not replicate easily in mammalian cells⁶. The RSV Gag protein, Pr76^{gag}, does not efficiently drive the budding process⁷ from mammalian cell membranes. We modified the Pr76^{gag} protein to enable it to function in mammalian cells by introducing a single point mutation in the protein, changing its second amino acid from Glu to Gly. The simple change creates a myristic acid addition site, which is required by the Gag proteins of almost all mammalian retroviruses⁸⁻¹¹. The change was sufficient to allow high levels of RSV particle formation in mammalian cells¹². With the modified RSV Gag protein (Pr76^{my}) mammalian cells produce enveloped particles with an exceedingly efficient half-time of 30 minutes, a rate that is faster than that of many mammalian retroviruses. The viral proteins within

these particles are identical in size to the mature Gag cleavage products of authentic RSV (ref. 13) and cleavage of Pr76^{myr} is mediated by the RSV protease located at the protein's C-terminus¹⁴. Truncating Pr76^{myr} to remove its protease activity does not impair assembly⁴. The minimum portion of the Pr76 N-terminus that is needed for assembly is under investigation.

To test the Gag-fusion method of retro-secretion, we coupled iso-1-cytochrome c from yeast to the C-terminus of Pr76^{myr} by fusing the two coding regions of the corresponding genes⁴. Iso-1-cytochrome c was chosen primarily due to the local availability of the gene (*CYCI*) and antibodies specific for the protein, but also because it contains a restriction site that allowed nearly the entire protein (105 of 108 amino acids) to be attached to Pr76^{myr} (ref. 15). Mammalian cells transfected with the chimaeric gene release into the medium particles containing a protein larger than Pr76^{myr} which reacts with anti-serum against RSV Gag proteins or against iso-1-cytochrome c. A rather unexpected outcome of the experiment was the release of cleavage products derived from the fusion protein.

Because the truncated Gag protein lacks the viral protease activity required for normal processing, the appearance of these cleavage products suggests that a cellular protease may have access to the fusion proteins during particle assembly. Further research will be required to determine if this additional protease activity can be recruited to separate precisely the foreign protein from Pr76^{myr} during retro-secretion. If not, then it may be practical to link the foreign protein to Gag with a synthetic protease cleavage site. Further work is also required to determine the packaging restrictions of this method, but it has been observed that very large proteins, such as the *pol* product of murine leukaemia virus, can impede the budding process if fused to the Gag protein¹⁶.

Glycoprotein packaging

Glycosylated proteins from higher eukaryotes cannot be made in prokaryotic cells because the latter do not possess the machinery required to synthesize and attach the proper oligosaccharide moieties. Even lower eukaryotic cells, such as yeast, may not provide carbohydrate chains sufficiently normal to endow the protein with the proper biological function. Thus, mammalian cells seem certain to remain important to the biotechnology industry for some time to come. Their use would be enhanced if glycoproteins of interest could be efficiently incorporated into the envelope of retroviral particles for easier purification. The ability to retro-secrete cellular glycoproteins is presently limited by a poor understanding of the molecular interactions involved in the packaging of

On the biotech beat

A gun for firing DNA into plant cells, a disposable bioreactor and hollow-fibre membrane purification systems top this week's offerings for biotechnology.

BRANSON Ultrasonics Corporation offers its Sonifier II line of **ultrasonic cell disruptors** for the ultrasonic disintegration and homogenization of cells for laboratory, pilot-scale or production use (*Reader Service No. 101*). Branson says the Sonifiers can handle most processes requiring high or low intensity ultrasonic energy. The ergonomically designed units feature a built-in broadband tuning circuit that eliminates manual retuning. The pulser mode applies ultrasonic energy to a solution at a rate of one pulse per second. The pulse duration can be adjusted from 0.1 to 0.9 seconds, enabling a solution to be processed at full ultrasonic intensity while limiting temperature. The Sonifiers come in a 200 W model for \$2,260 (US) and a 400 W model for \$2,590–2,610 (US). Available optional equipment includes micro-tips, flow-through horns, cup horns, sealed atmosphere chambers,



Branson's Sonifier II disruptor.

rosette cells, tissue disruptors and sound-proof boxes.

Fisher Scientific's 16-channel **bioelectrode scanner/controller** provides round-the-clock monitoring and regulation of experimental processes, and has particular application in fermentation reactions (*Reader Service No. 102*). The Accumet 935 Bioelectrode Scanner/Controller has a scanner module that accepts pH, ion-selective, redox, reference and combination electrodes, and can scan in 0.3 seconds per enabled channel, says the company. Experimental conditions are regulated by the controller module via pumps, heaters, motors, shakers and dispensers. The system is interfaced with a personal computer, and the user-friendly software allows the operator to store data on diskette, view electrode conditions on a monitor, and develop protocols for the standardization of experimental conditions. Prices start at \$9,636 (US).

Large-volume Western blotting is now possible using Integrated Separation Systems' MEGA System (*Reader Service No. 103*). MEGA DALT-20 is capable of blotting nine full-size gels or running up to 20 SDS-PAGE gels simultaneously, with an efficient use of time, space and materials, says the company. The integrated system can also be used for isoelectric focusing. The company supplies all the components for the system, including pre-

retroviral *env* products.

Packaging has been hypothesized to involve an interaction between residues within the transmembrane region of retroviral glycoproteins and portions of the Gag protein¹⁷. The packaging rules are probably similar among retroviruses because it has been shown recently that Moloney murine leukaemia virus can incorporate the glycoproteins from some other retroviruses¹⁸. Furthermore, preliminary data suggest that the haemagglutinin (HA) from influenza virus can be packaged into RSV virions when the transmembrane and cytoplasmic domains of HA are replaced with those of RSV (Eric Hunter, personal communication). These results contribute to the excitement surrounding the development of retro-secretion and the further domestication of retroviruses in general. □

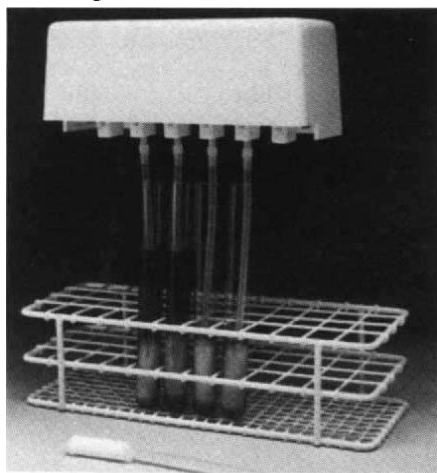
John W. Wills is at the Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center—Shreveport, 1501 Kings Highway, P.O. Box 33932,

Shreveport, Louisiana 71130, USA. For more information, fill in reader service number 100.

- Smith, R.E. *Meth. Enzymol.* **58**, 393 (1979).
- Dickson, C., Eisenman, R., Fan, H., Hunter, E. & Teich, N. in *RNA Tumor Viruses Vol. 1* (eds Weiss, R., Teich, N., Varmus, H. & Coffin, J.) 513–648 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984).
- Voynow, S.L. & Coffin, J.M. *J. Virol.* **55**, 79 (1985).
- Weldon, R.A., Erdie, C.R. & Wills, J.W. (in preparation).
- Adams, S.E., Dawson, K.M., Gull, K., Kingman, S.M. & Kingman, A.J. *Nature* **329**, 68 (1987).
- Weiss, R. in *RNA Tumor Viruses Vol. 1* (eds Weiss, R., Teich, N., Varmus, H. & Coffin, J.) 209–260 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984).
- Vogt, V.M., Bruckenstein, D.A. & Bell, A.P. *J. Virol.* **44**, 725 (1982).
- Schultz, A.M., Henderson, L.E. & Oroszlan, S. *Ann. Rev. Cell Biol.* **4**, 611 (1988).
- Towler, D.A., Gordon, J.I., Adams, S.P. & Glaser, L. *Ann. Rev. Biochem.* **57**, 69 (1988).
- Rein, A., McClure, M.R., Rice, N.R., Luftig, R.B. & Schultz, A.M. *Proc. natn Acad. Sci. U.S.A.* **83**, 7246 (1986).
- Rhee, S.S. & Hunter, E. *J. Virol.* **61**, 1045 (1987).
- Wills, J.W., Craven, R.C. & Achacoso, J.A. *J. Virol.* (in the press).
- Leis, J. *et al. J. Virol.* **62**, 1808 (1988).
- Skalka, A.M. *Cell* **56**, 911 (1989).
- Smith, M., Leung, D.W., Gillam, S. & Astell, C.R. *Cell* **16**, 753 (1979).
- Felsenstein, K.M. & Goff, S.P. *J. Virol.* **62**, 2179 (1988).
- Perez, L.G., Davis, G.L. & Hunter, E. *J. Virol.* **61**, 2981 (1987).
- Wilson, C., Reitz, M.S., Okayama, H. & Eiden, M.V. *J. Virol.* **63**, 2374 (1989).

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Millipore says its new low-protein-binding Immersible-CX Ultrafilter offers a faster, gentler, more convenient method



Millipore's filter for small volumes.

than stirred cells for **concentrating and desalting** 1–100-ml volumes of protein or virus (*Reader Service No. 104*). The device incorporates the low-binding, regenerated cellulose PLGC ultra-filtration membrane, which is designed for maximum recoveries. The self-contained Immersible-CX unit eliminates preparation and clean-up steps, without the need to transfer, says Millipore. Up to six units can be operated simultaneously to concentrate 1–100-ml volumes in each test tube. The system's gentle agitation will not damage shear-sensitive enzymes or virus, says the company. The reusable 100 ml Immersible-CX Ultrafilter sells for \$3.80 each (US).

Transfection tools

Du Pont is now selling a **transfection gun** for shooting microparticles coated with DNA and other genetic substances through the tough cell walls surrounding plant cells without killing them (*Reader Service No. 105*). The Biolistic gene gun fires a 22-calibre cartridge that propels a large plastic projectile loaded with millions of small tungsten particles coated



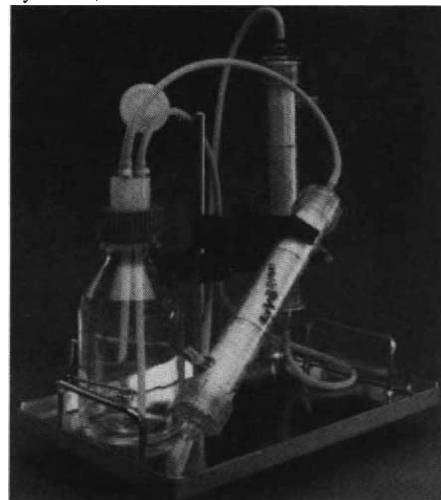
The Du Pont Biolistics gene gun.

with genetic materials. The gun is enclosed in a stationary chamber, and has a special stopping plate to halt the large projectile while permitting the microparticles carrying genetic material to enter plant cells placed inside the chamber. The Biolistics gun was developed by researchers at Cornell University in New York (see *Nature* 327, 70; 1987). Du Pont is now leasing the gun for \$30,000 (US) plus royalties on sales of any new plant products developed with the gun.

Designed as a universal **cell permeabilizer**, the Biojet MI from B. Braun Medical Ltd uses electrical pulses of high intensity and short duration to increase the permeability of cell membranes, while preserving membrane integrity and cellular function, says the company (*Reader Service No. 106*). The microprocessor-controlled pulse generator simplifies the loading of living cells with molecules such as proteins, DNA, pharmaceutical substances and dyes, and facilitates cell fusion and DNA transfection. The cell chamber is filled under sterile conditions and can hold fluid volumes of 0.1–6.0 ml; temperature is maintained by a Peltier element. The menu-driven display leads the operator through the 20 available programs, which can consist of one to 25 operations. The pulse generator can operate in manual-pulse mode where single or multiple pulses are of predetermined duration and voltage, or in auto-pulse mode where they may be preprogrammed.

Cultural assets

The Network 1000 system from Kinetek Systems, Inc. is a **hollow fibre cell culture**



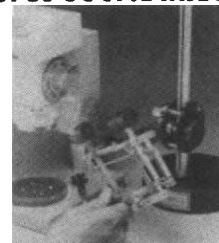
Disposable bioreactor from Kinetek Systems. starter kit for protein production (*Reader Service No. 107*). The \$1,975 (US) kit has been developed specifically for the research-level production of proteins excreted from the culture of mammalian and insect cells. Central to the system are Kinetek Systems' Hybrinet hollow fibre bioreactor and gas exchange module hollow fibre oxygenator cartridges, which

the company says permit highly efficient nutrient and oxygen transfer and product removal in a continuous perfusion mode without stirring or mixing mechanisms. The kit's entire hydraulic circuit can be autoclaved once it is assembled on its electropolished tray, before seeding with cells and placing it in an incubator. Kinetek Systems also offers its hollow fibre bioreactor as a microprocessor-controlled system for \$8,300 (US).

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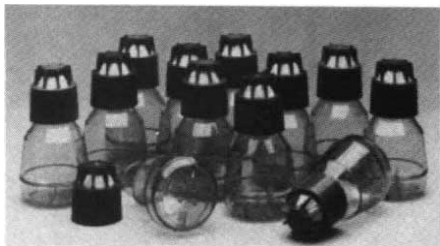
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Research Products International has specially designed **fermentation flasks** for



RPI's Tunair shaker flasks.

use with conventional shaker platforms (*Reader Service No. 108*). The company says its Tunair Shake-Flasks provide optimum culture conditions in aerobic fermentation, screening of antibiotics and secondary metabolites, fermentation optimization and scale-up studies. The flasks are available in two designs to achieve a large range of aeration values and shear levels. Baffles at the bottoms of the flasks simulate the mass transfer action of impeller-agitated fermenters. The flasks have slip-on closures with gauze filter linings, and barrel-shaped trunks to increase the vortex surface. The working volume of the 300 ml flask is 60 ml. The flasks come six to a case for \$75-96 (US).

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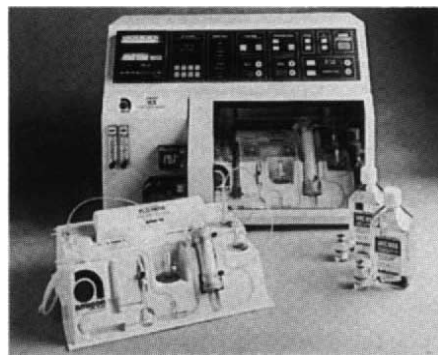
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Reader Service No.25

A new addition to Applikon's range of cell culture vessels is the 7-litre multiport **cell culture bioreactor** (*Reader Service No. 109*). The multiport system enables the bioreactor to be customized to suit individual needs. The bioreactor has 19 ports that can be used for bubble-free aeration of the cell culture, or for inserting sensors for pH, temperature, CO₂, dissolved oxygen and antifoaming solutions. Agitator systems, using either mechanical or magnetic stirrers, ensure the uniform distribution of nutrients and trouble-free operation during long-term continuous fermentations, says Applikon. The round-bottomed autoclavable bioreactor vessel is 550 mm high and 220 mm wide.

A fully automated system for high-density cell culture and **monoclonal anti-**

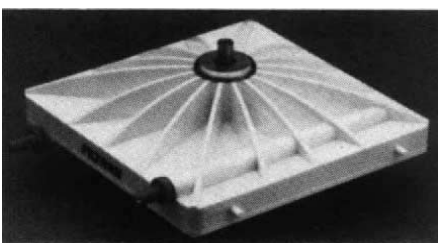


Amicon's Vitafiber VLS for MAb production.

body production is available from Amicon (*Reader Service No. 110*). The Vitafiber VLS system comes as a compact benchtop unit complete with Vitafiber Flo-Path bioreactor, incubator, pumps, controls, probe and six-channel recorder. The system offers on-line monitoring and regulation of cell culture conditions, including pH, temperature, medium feed and recirculation rates. Other features include touch-pad control, digital readouts and quick connect/disconnect fittings to simplify use. The hollow-fibre bioreactor comes as a ready-to-use disposable unit and is available with cartridges of 1,400, 3,700 and 7,000 cm² lumen area; it can be used in incubators and warm rooms, or as part of Amicon's integrated cell culture system.

Product purifiers

The Ultrasette tangential flow **ultrafiltration or microfiltration devices** from Filtron Technology Corporation are designed for



Tangential filtration from Filtron.

the rapid and efficient separation, concentration or diafiltration of antibodies, enzymes, cells, viruses and other biological solutions (*Reader Service No. 111*). The 1-ft² membrane of the self-contained units is encapsulated in a plastic cell ready to connect directly to benchtop tubing pumps. Each unit is tested for total integrity, says the company. The Ultrasettes come with membranes from Filtron's low-binding PES Omega Series, with ultrafiltration cutoffs of 1K, 3K, 5K, 8K, 10K, 30K, 50K, 100K, 300K and 1,000K, for \$590-700 (US). Microporous membranes include 0.16 µm and 0.3 µm membranes.

NY Gene Corp. says its 1-gram **MASS membrane affinity separation system** can separate up to 10 grams per hour of monoclonal antibody from serum, ascites and cell culture supernatant, with a purity of more than 98 per cent (*Reader Service No. 112*). The reusable MASS device incorporates a microporous membrane covalently bound to recombinant Protein A, resulting in highly stable ligand coupling and low non-specific binding, says the company. It can be used to purify polyclonal as well as monoclonal antibodies, including all mouse IgG subclasses.

Sepragen Corporation offers a computer-controlled, low-pressure **biopurification system** for use in large-quantity bioseparations (*Reader Service No. 113*). The Quanta Sep-750 can perform chromatographic separations of up to 750 millilitres per minute in columns ranging in volume from 100 millilitres to 20 litres, says the company. The menu-driven system provides built-in flexibility in terms of the selection of separation parameters, which include multi-solvent selection and fraction collection. The system offers a choice of separation menus, including both gradient and step elutions. The need for repetitive programming is eliminated as the operator is able to create and store specific separation protocols. The unit is biocompatible and has standard flow, UV, and conductivity detection; it also comes with sensors for fluid and pressure.

Memtek has expanded its line of cartridges used for **process-scale affinity purification** (*Reader Service No. 114*). The company's range of megaMAC Cartridges are now available prebound with recombinant protein A and protein G; mouse, human or rat IgG; or ready for immobilization with protein ligands such as antibodies, haptens, amino acids, and other macromolecules. □

These notes are compiled by Diane Gershon and Carol Ezzell from information provided by the manufacturers. To obtain additional information about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.